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CHEMICAL CONSTITUENTS OF AMARANTHUS RETROFLEXUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 254

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(WITH ELEVEN FIGURES)

Introduction

It is a well known fact that weeds retard the development of cultural plants. This is due to a number of causes: use of water, shading, use of nutrient salts, etc. It has been claimed for various species of *Amaranthus* that they not only absorb nitrates to care for their nutrient needs, but that they store much nitrogen as nitrate. If this be true, this genus has an excellent adaptation to enable it to combat cultural plants, for nitrate supply is a common limiting factor for crop growth. In order to investigate this statement, to locate the place of nitrate storage, and to determine the amount of nitrogen used otherwise by this plant, separate analyses were made of roots, stems, leaves, and branches of *Amaranthus retroflexus* at various stages and under various conditions of growth. The amount of the several carbohydrates was also determined in each analysis, in order to calculate the carbohydrate-nitrogen ratio which is lately receiving so much attention. A tissue analysis of the seeds was also made in an endeavor to ascertain more fully the chemical constituency of this plant, with the hope of learning more of the peculiar germinative behavior of these seeds.

Historical

The first chemical analysis of *Amaranthus* was made by BOUTIN (1), a French chemist, in 1873. Housewives of that time were using these plants to clean their cooking utensils. This fact gave it some commercial importance and brought it to the attention of chemists. It was thought that the ability to "cut grease" must be due to acids in the plant. To verify this BOUTIN incinerated 100 gm. dry weight of the entire plant, and obtained 16 gm. residue. Water was added to leach out the soluble salts. The soluble portion weighed 8 gm. He called this potassium carbonate, and calculated the equivalent weight in grams of potassium nitrate, and found it to be 11.68, or 11.68 per cent. BOUTIN concluded that this plant was neutral in reaction on account of the presence of the neutral salt KNO_3 (as a matter of fact this plant is acid in reaction). Later (2) he made analyses of the other species of *Amaranthus* by the same method, to determine the amount of KNO_3. The result of his analysis was as follows: *A. atropurpureus* contains 22.77 per cent KNO_3 (one kilogram gives 31 gm. N and 103.5 gm. K); *A. Blitum* contains 11.68 per cent KNO_3; *A. ruber* contains 16 per cent KNO_3 (one kilogram gives 22 gm. N and 72 gm. K). It is evident that BOUTIN's method is not an accurate quantitative method of determining the amount of nitrate present. On the other hand, he demonstrated by other qualitative methods that the several species of *Amaranthus* studied contain a large amount of potassium nitrate. BROSSET (3) suggests the use of these plants as a fertilizer.

PAMMEL and DOX (15), in 1917, made microchemical tests of three common pigweeds, *A. blitoides*, *A. graecizans*, and *A. retroflexus*, and found them to contain abundant starch, some protein, and a little fat. In addition they made the Kjeldahl-Gunning nitrogen determination and found these species to have 1.88, 2.32, and 2.49 per cent of nitrogen. Multiplying by the factor 6.25, they obtained 11.75, 14.52, and 15.59 per cent of protein respectively.

HARDING and EGGE (8) made an analysis of the seeds of *A. retroflexus* for fats, protein, starch, sugars, hemicellulose, crude fiber, and tannin.

Of late much significance is being attached to the carbohydrate-nitrogen ratio of plant tissues, or, as FISCHER (6) puts it, the $\frac{C}{N}$. On the basis of work done by him and others, FISCHER makes the following generalizations. If the value of $\frac{C}{N}$ rises by an increase in the amount of carbon, or by a decrease in the amount of nitrogen furnished the plant, there is an increase in the amount of flowering. If the value of $\frac{C}{N}$ drops by a decrease in the amount of carbon, or by an increase in the amount of nitrogen furnished the plant, there is an increase of vegetative growth and a reduction of flowering. Briefly stated, a great preponderance of carbohydrates in plants favors flowering. Since the carbon of plants is fixed from the carbon dioxide of the air by photosynthesis, conditions that favor photosynthesis will tend to increase the ratio, and according to FISCHER the flower production. He found that increased partial pressures of carbon dioxide in the air had this effect. Since nitrogen is absorbed from the soil in the form of nitrates, conditions that favor nitrate absorption will decrease the ratio, and according to FISCHER favor vegetation.

KRAUS and KRAYBILL (12), on the basis of much more critical work, including numerous cultures, tissue analyses, and microchemical and anatomical studies, conclude that a very high $\frac{\text{carbohydrate}}{N}$ value gives little vegetation and little or no reproduction; a medium $\frac{\text{carbohydrate}}{N}$ value gives moderate vegetation and good reproduction; and a low $\frac{\text{carbohydrate}}{N}$ value gives vigorous vegetation and little reproduction. Through their extreme conditions of culture, withholding nitrates, it is probable that KRAUS and KRAYBILL got much higher carbohydrate plants than FISCHER obtained in his cultures, hence their conclusion that very high $\frac{\text{carbohydrate}}{N}$ gives little vegetation or reproduction. In short, FISCHER worked only on the portion of the $\frac{C}{N}$ curve that induced fair vegetation and good reproduction or extreme vegetation and little reproduction, but not on the extreme of the curve that greatly

reduces both vegetation and reproduction. KRAUS and KRAYBILL cite literature showing that various conditions that greatly retard growth produce high carbohydrate plants. It seems that such conditions retard the use of carbohydrates for building new tissue to a greater degree than they do photosynthesis, and thereby lead to an accumulation of carbohydrates.

HEDLUND (9) finds that under like cultural conditions those varieties of winter wheat that have a higher percentage dry weight in the autumn are generally more winter hardy than the ones having a low percentage dry weight, and that cultural conditions that make for high percentage dry weight in any variety also make for winter hardiness. He finds, as do KRAUS and KRAYBILL, that high percentage dry weight is due to high percentage carbohydrate, and therefore high $\frac{\text{carbohydrate}}{\text{N}}$.

RIBERA (16) finds that all cultural conditions that increase the percentage dry weight in wheat decrease lodging. From this and the two investigations previously mentioned it is evident that high $\frac{\text{carbohydrate}}{\text{N}}$ increases straw strength and decreases lodging. High percentage of carbohydrate is said to increase hardiness, at least in part, by the greater amount of glucose present, and it may increase straw strength by inducing greater development of mechanical tissue along with greater thickness of walls, as KRAUS and KRAYBILL found for certain tissues of the tomato.

Methods and results

GREEN PLANT AT VARIOUS STAGES OF DEVELOPMENT

Preparation of samples.—Samples were secured on June 3, June 20, and July 8 consecutively from a vacant lot on 59th Street and Ingleside Avenue, Chicago. On June 20 samples were taken from two places, namely, the manure pile (rich soil) and the knoll (poor soil) for comparative work. The soil particles adhering to the roots and rootlets were removed by running water from a filter pump. As the velocity of water was very great, the soil particles were removed without difficulty. The roots were partially dried by the air current from the laboratory air line, and

finally dried by the use of paper towels. The roots, stems, and leaves were detached for separate analysis. The roots and the main stems were separated by cutting just between the cotyledon scar and the first branching rootlet. The leaf blades with the petioles were separated from the stem. Each portion was weighed and the length and diameter measured. Table I gives a brief description of the three consecutive samples.

TABLE I

THE GREEN PLANT AT VARIOUS STAGES OF GROWTH

Measurement	June 3 collection	June 20 collection	July 8 collection
	Inches	Inches	Inches
Average height	1.00–4.00	6.00–8.00	20.00
Taproot length	1.00–1.50	4.00–6.00	4.00– 6.00
Taproot diameter	.....	0.10–1.00	1.00– 1.20
Secondary rootlets length	None	0.10–1.00	8.00–14.00
Secondary rootlets diameter	None	.....	0.13– 0.25
Stems length	1.00–4.00	6.00–8.00	20.00
Stems diameter	0.13–0.25	0.37–0.75	0.13– 0.25
Lateral branches length	None	0.10–1.50	14.00
Lateral branches diameter	None	.....	0.25– 0.50
Seed head	None	None	0.25– 1.00
Green weight	Grams	Grams	Grams
Roots	26.00	28.50	29.20
Main stems	52.05	97.40	88.30
Branches	None	None	80.45
Leaves	188.90	142.20	110.25

The green samples were then immediately put in a freezing chamber, allowed to freeze overnight, and ground in a meat grinder the next morning. The freezing prevents losses. In the frozen condition no juices ooze out or spatter in the manipulation. The samples were boiled with 95 per cent alcohol to destroy the enzymes, and were then transferred to extraction cups, with filter paper thimbles, previously dried and weighed. The tissues were fractionated according to KOCH's (11) scheme for tissue analysis, namely, the lipin or ether soluble fraction (F_1), the alcohol water soluble fraction (F_2), and the insoluble fraction (F_3). In the green plant F_2 was comparatively small, consisting of chlorophyll and extracts of various pigments. The F_1 was put together with F_2 for the following carbohydrate and nitrogen estimation.

ANALYSES

Nitrogen compounds

NITRATES.—The nitrates were determined by the Schlösing-Wagner method (**14**) as modified by KOCH for use in his laboratory. The modification consists essentially in the use of an inverted burette instead of a tube sealed at one end, and of the Van Slyke apparatus (only volumetric tube and Hemple pipette), to measure the true volume of nitric oxide. The principle of the method is: $3Fe^{++}+NO_3^-+4H^+ \rightarrow 3Fe^{+++}+NO\,\text{gas}+2H_2O$. Therefore 1 mol. NO gas gives an equivalent of 62 gm. of NO_3, or 1 cc. of $\text{gas}=\frac{62}{22400}=2.77$ mg. NO_3.

In order to determine the accuracy of this method, a known solution of KNO_3 (0.5 per cent) was used. Four consecutive determinations with 10 cc. of the known solution were made. The average volume of nitric oxide gas for each 10 cc. solution, calculated to standard condition, was 11.12 cc. The theoretical volume for 10 cc. of 0.5 per cent KNO_3 is 11.078 cc.

The determination of nitrates in the samples was made by taking an aliquot of the soluble fractions (F_1 and F_2). The nitric oxide gas driven over was caught in an inverted burette which had previously been filled with 40 per cent NaOH to absorb the CO_2 and neutralize the hydrochloric acid (HCl gas will come over when the hydrochloric acid concentration reaches 20 per cent in the boiling flask). The burette containing the nitric oxide gas was set aside and allowed to cool to room temperature; then the nitric oxide gas was transferred to the Van Slyke apparatus. The total volume and the volume of unabsorbed gas were recorded. The absorbed volume by the alkaline $KMnO_4$ in Hemple pipette is that of nitric oxide. This volume was then calculated to standard volume from temperature and barometric pressure, for example:

	I	II
Aliquot in cc. used	25.0	25.0
Total volume of gas (nitric oxide+air)	4.80	4.85
Volume of unabsorbed gas (air)	1.27	1.26
Volume of (absorbed) nitric oxide	3.53	3.59
Barometric pressure=746.9; temperature 20.5° C.		
Volume at standard condition	3.23	3.27

	I	II
Equivalent in milligrams of NO_3	8.95	9.06
Milligrams of dry substance (25 cc.) used	107.75	107.75
Percentage NO_3 in soluble fractions F_1+F_2	8.30	8.41
The percentage soluble fractions in whole samples	45.9	45.9
Therefore percentage of NO_3 calculated on whole sample	3.81	3.86

Soil samples were taken at the same time that the green plants were gathered. The nitrates were estimated by the colorimetric method with phenoldisulphonic acid. The moisture in percentage and nitrates in parts per million are shown in fig. 1.

On June 20 the samples from the manure pile and from the knoll were taken for comparison. The nitrate content of the soil and that in the plant were as follows:

	Knoll sample	Manure pile sample
NO_3 in ppm in soil	300	29
NO_3 in percentage in plant (stem only)	1.71	1.45

The high NO_3 content in the soil of the knoll sample is probably due to the fact that some one, perhaps the gardener, had disturbed the soil by dragging his cultivator over it accidentally in cultivating his plot near by. The second reason is the better drainage and aëration in the knoll, and therefore better conditions for nitrification; but the striking fact is that high nitrate content in the soil did not bring about a proportional high nitrate content in the green plant organs. The rate of absorption increases with the aging of the plant; when the plants were about 25 days old, the nitrate in the stem was only 1.71 per cent. Eighteen days later (July 8) the nitrate content had risen to 8.58 per cent. During the same period branches grew from 0.1 to 14 inches, and their nitrate content rose to 12.50 per cent. This rapid increase in absorption of nitrates may partially be explained by the increase in extent of the absorbing roots from a radius of a few inches to about 2 ft.

The nitrate content in the roots, stems, and leaves is given in table II and is also shown in fig. 1. The nitrate content of the roots falls gradually from 1.85 per cent on June 3 to zero on June 20. At the same time nitrates in the leaves fall from 1.38 per cent to zero, while in the stem there is a gradual increase. There must be a definite reason for such differences. The differences may be due

to many causes. In the leaves protein synthesis is going on continuously in the presence of soluble carbohydrates. There is also synthesis of other organic nitrogen compounds, such as chlorophyll,

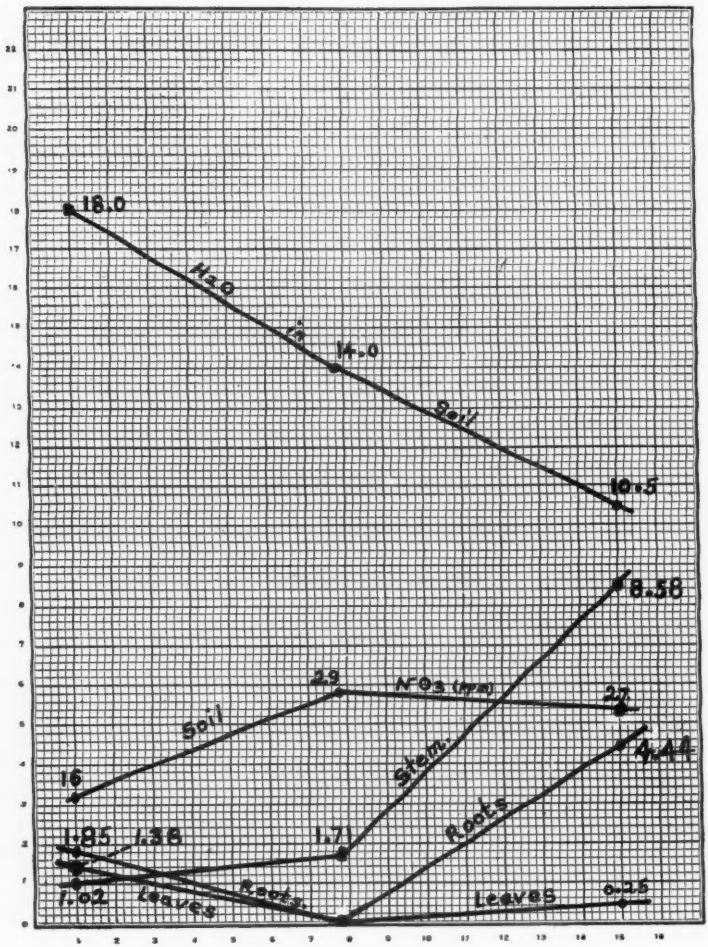

FIG. 1.—Relation of soil nitrification and nitrate intake by green plant organs; nitrate in soil expressed in parts per million; all other data calculated as percentage on dry weight basis.

phospholipins, etc., and all of the nitrates of the leaves seem to be used up in these syntheses. The nitrates are carried from the roots to other parts of the plants as fast as they are taken up from the soil. There may be as high a concentration of nitrates in the roots as in the soil (29 parts per million). This low concentration cannot be estimated by this method and would therefore be missed.

The stem and branches are the primary nitrate storage organs. The nitrate content rises as high as 8.58 per cent in the stem and 12.5 per cent in the branches during the early seed formation period. This high content is shown still more clearly by the ratio of nitrate nitrogen to the total nitrogen. This is 32.8 per cent for the roots, 51.85 per cent for the stems, 56.4 per cent for the branches, but only 1.25 per cent in the leaves. Curves showing this ratio in these organs at different stages are given in fig. 2. This large supply of nitrates in the stem and branches may be drawn upon heavily for further growth and seed production, although the supply seems more than adequate for these uses. There is also no reason for thinking that nitrate absorption ceases at this time. The extent to which this storage of nitrate is drawn upon by later development could be ascertained by the analysis of a set of samples taken late in the fall when seed formation and growth were complete. It is to be regretted that circumstances made such an analysis impossible for this paper.

It is worthy of note that the nitrate storage organs are the ones that made the most rapid growth in length, weight, and volume. The stem which rose from 8 inches on June 20 to 20 inches on July 8 at the same time increased in nitrate content from 1.71 to 8.58 per cent on dry weight basis. In addition to the stem there are numerous side branches which elongated from 0.1 to 14 inches in 18 days, making nearly 1 inch in 24 hours. At the same time there was an increase in percentage of nitrates per gram of dry matter from 1.71 on June 20 to 12.50 per cent on July 8. The rate of nitrate intake per gram per hour seems to follow a geometrical progression in each individual plant.

It appears that *Amaranthus* may be a very considerable factor in depleting soils of their nitrates. Also in case the weeds are burned the nitrogen stored is permanently lost from the soil. It

is a point of interest to know how generally this great power of absorbing and storing nitrates is possessed by weeds.

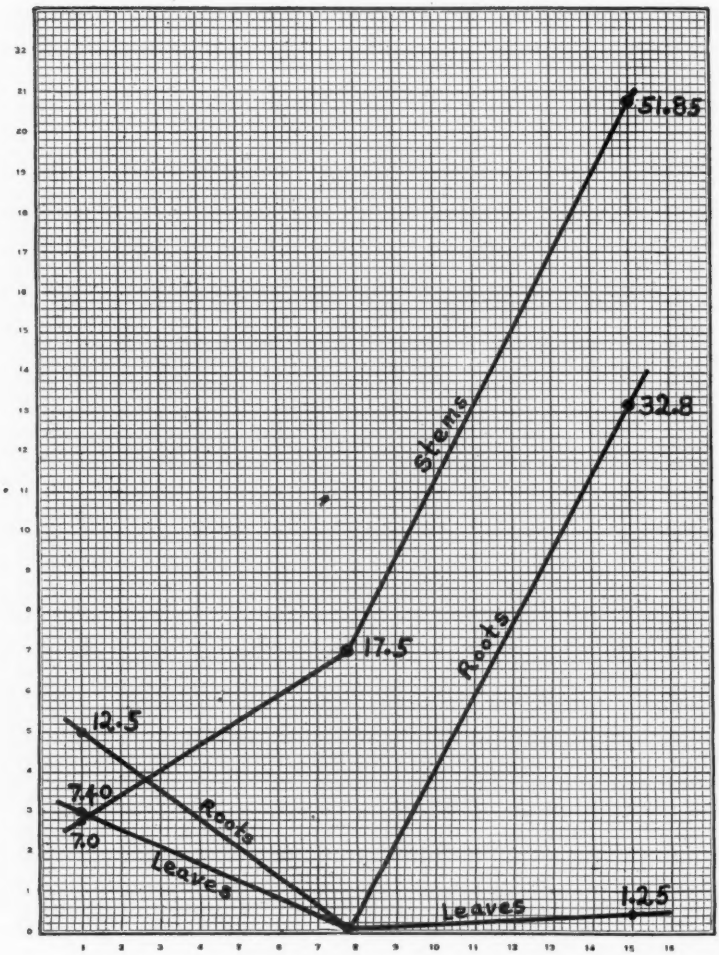

FIG. 2.—Ratio of nitrate nitrogen to total nitrogen; ratio for branches (not shown in figure) 56.4.

AMINO N.—The amino nitrogen was determined by the Van Slyke apparatus. The amino acids thus determined are chiefly

of the mono-amino-monocarboxylic acids. In each estimation only 2 cc. of the solution was used. The amino acid nitrogen deter-

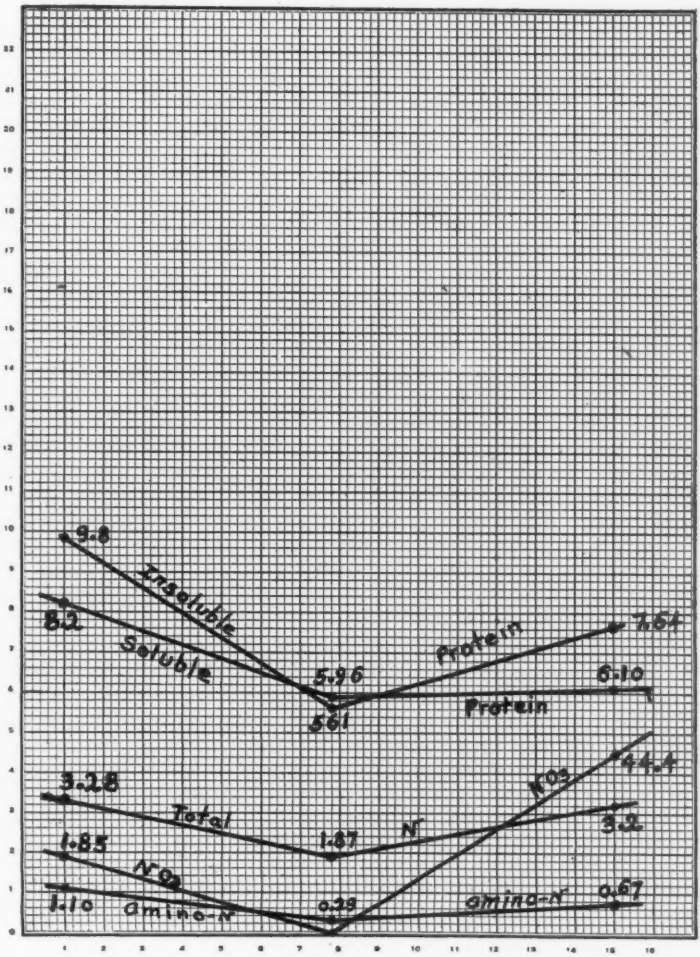

FIG. 3.—Nitrogen compounds in roots

mined throughout the season is given in table II. Curves showing the variation in roots, stems, and leaves are given in figs. 3, 4, and 5 respectively. In all the three organs the variation is very small.

In general the amino N varies directly with the insoluble protein; with a high protein content, high amino N; and low protein content, low amino N.

TABLE II

THE NITROGEN COMPOUNDS IN THE GREEN PLANT

Material	Roots		Stems		Branches		Leaves	
	June 3 collection (1–4 inches)							
Total N	3.33	3.33	3.19	3.21	....	....	4.25	4.27
Nitrates NO_3	1.77	1.93	0.99	1.04	....	....	1.36	1.40
Nitrate N	0.40	0.43	0.22	0.23	....	....	0.31	0.32
Amino N	1.09	1.13	0.43	0.43	....	....	0.97	0.96
Insoluble N	1.62	1.52	1.74	1.73	....	....	3.21	3.23
Insoluble protein	10.18	10.25	10.92	10.80	....	....	20.20	20.30
Soluble protein	8.24	8.17	7.67	7.92	....	....	4.58	4.52
	June 20 collection (6–8 inches)							
Total N	1.85	1.88	2.39	2.21	....	....	4.56	4.51
Nitrates NO_3	None	None	1.67	1.75	....	....	None	None
Nitrate N	None	None	0.38	0.40	....	....	None	None
Amino N	0.29	0.29	0.19	0.19	....	....	1.38	1.37
Insoluble N	0.86	0.91	0.95	0.97	....	....	3.66	3.59
Insoluble protein	5.40	5.72	5.86	6.10	....	....	23.00	22.6
Soluble protein	5.92	6.10	5.28	5.41	....	....	5.66	5.78
	July 8 collection (20 inches)							
Total N	3.21	3.12	3.74	3.80	5.04	4.94	4.85	4.80
Nitrates NO_3	4.41	4.47	8.40	8.75	12.50	12.40	0.25	0.246
Nitrate N	0.99	1.02	1.90	1.98	2.85	2.80	0.06	0.06
Amino N	0.63	0.70	0.35	0.34	0.48	0.49	1.44	1.42
Insoluble N	1.29	1.14	0.95	0.91	1.01	0.98	3.29	3.21
Insoluble protein	8.10	7.17	5.96	5.71	6.35	6.15	20.62	20.08
Soluble protein	6.16	6.05	5.60	5.70	7.42	7.30	9.42	9.62

INSOLUBLE PROTEIN.—The insoluble protein was calculated from nitrogen of the insoluble fraction (F_3). The insoluble protein is given in table II, and curves showing fluctuation during the growing season are given in figs. 3–5. The insoluble protein falls and then rises again at maturity in the root, while in the leaves the fluctuation is in the opposite direction (see curves). In the stem the decline is in the early stage from 10.89 per cent (June 3) to 6.03 per cent (June 20). From that time on the curve is almost a straight horizontal line; therefore the rate of synthesis of the insoluble protein must have been keeping pace with the growth of the stem,

because the fall at this time is only from 6.03 to 5.84 per cent (almost within experimental error).

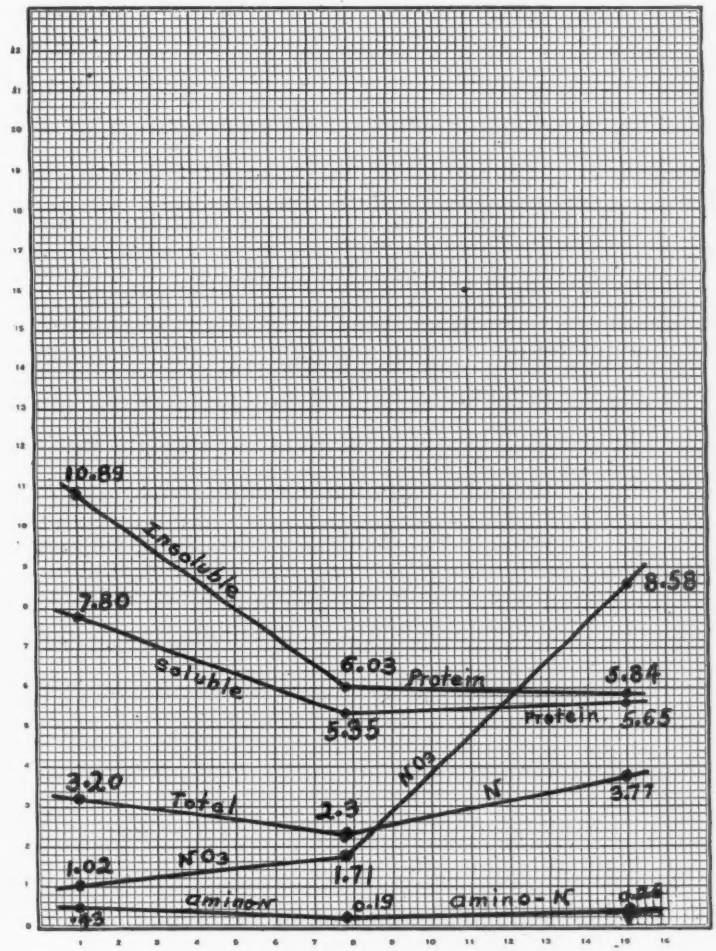

FIG. 4.—Nitrogen compounds in stems

SOLUBLE PROTEIN.—Soluble protein in F_1 and F_2 is computed from the Kjeldahl nitrogen determination. The nitrate nitrogen

was determined separately (by the method previously described); so the Kjeldahl determination was conducted without any modifica-

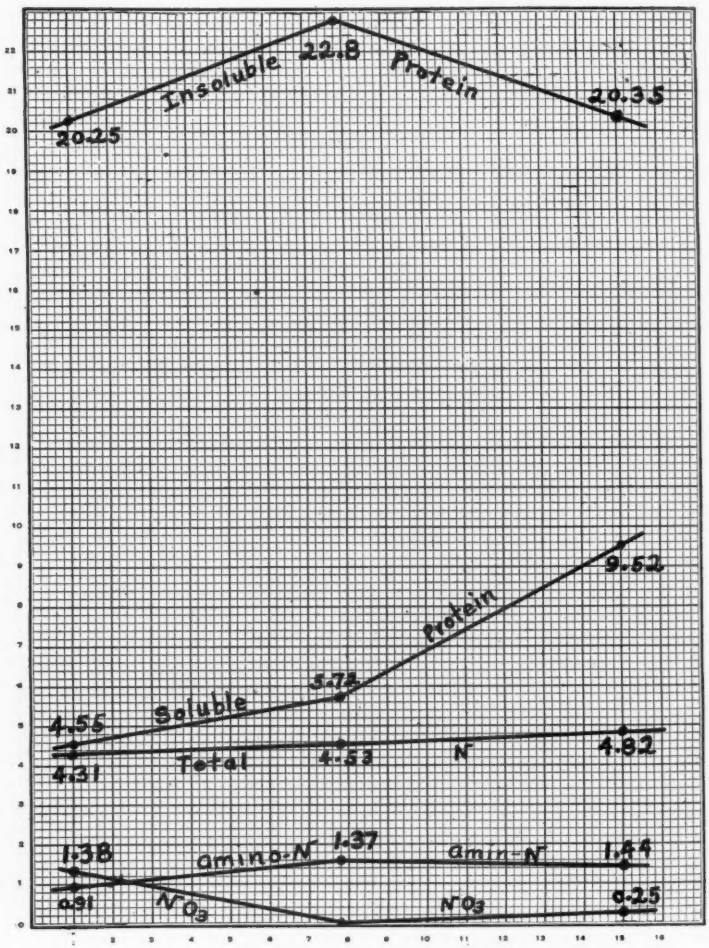

FIG. 5.—Nitrogen compounds in leaves

tion for nitrates. (Previously zinc was used, but this did not reduce any nitrate. Experiment with a known solution 0.5 per cent KNO_3,

per official method by the use of salicylic acid and sodium thiosulphate, reduced only 60 per cent of the nitrate into ammonia.)

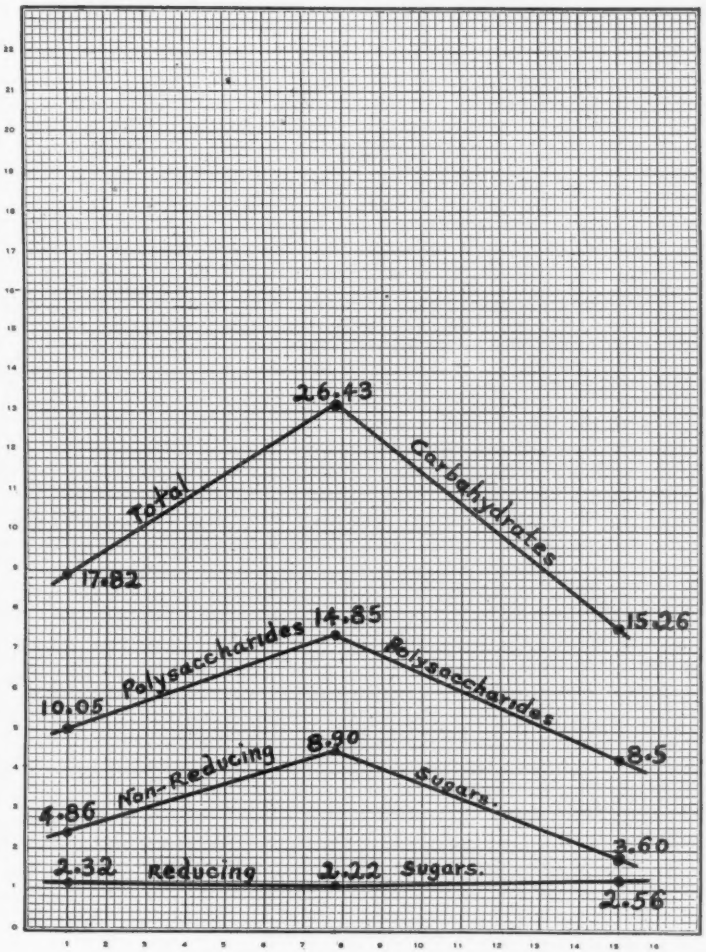

FIG. 6.—Different carbohydrates in roots

It is incorrect to call all this nitrogen as derived from protein, because part of the soluble nitrogen was from the breaking down

of the chlorophyll; but for comparison it is not out of place to calculate the N by the factor 6.29 to convert it into soluble protein

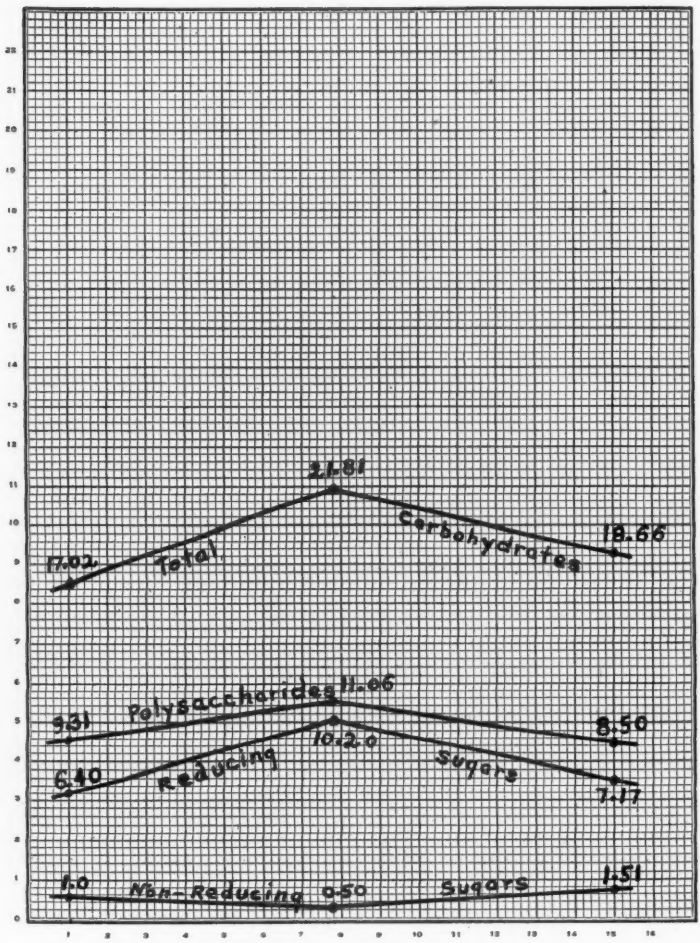

FIG. 7.—Different carbohydrates in stems

for temporary convenience in interpreting the results. The curves of the soluble protein in figs. 3–5 are self-explanatory, showing the variation throughout the season.

CARBOHYDRATES.—The carbohydrates were determined by the reduction method with Fehling solutions. The cuprous oxide

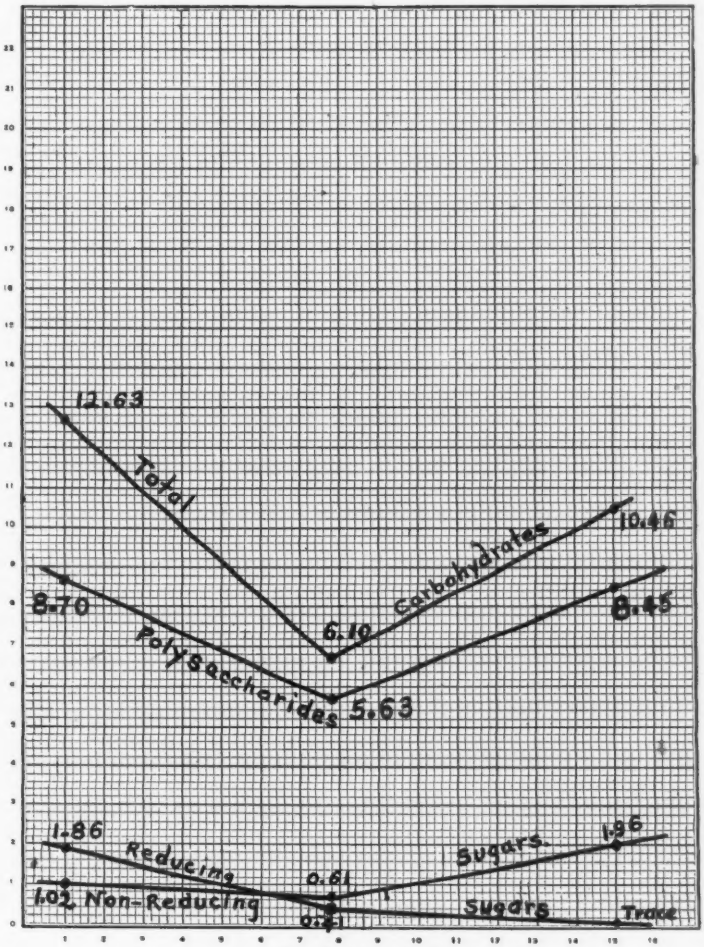

FIG. 8.—Different carbohydrates in leaves

obtained was dissolved in excess of ferric ammonium sulphate with H_2SO_4 previously added. The ferrous ions produced by the oxidation of cuprous oxide were titrated against a N/20 $KMnO_4$

solution, in which 1 cc. represents 3.1 mg. of copper. The corresponding equivalents of the different sugars expressed in milligrams were found in the Munson-Walker table. The weight of sugar found divided by the material used gives the amount of sugar contained in 1 gm. of material.

The soluble carbohydrates are in F_1 and F_2. The reducing sugar was first determined. The non-reducing sugar was obtained by subtracting the reducing sugar from the total sugar by hydrochloric acid hydrolysis at 67–69° C. for 10 minutes.

The insoluble carbohydrates are in F_3. They consist essentially of colloidal polysaccharides, the greater part of which was starch. The polysaccharides were determined by the Fehling solution after acid hydrolysis for 2.5 hours with a reflex condenser.

TABLE III

THE CARBOHYDRATES IN THE GREEN PLANT

Material	Roots		Stems		Branches		Leaves	
	June 3 collection (1–4 inches)							
Total carbohydrates	17.81	17.83	16.95	17.09	....	.....	12.76	12.57
Reducing carbohydrates	2.38	2.27	6.51	6.30	....	.....	1.86	1.87
Non-reducing	5.43	5.46	1.16	1.44	....	.....	2.14	2.05
Polysaccharides	10.00	10.10	9.28	9.35	....	.....	8.76	8.65
	June 20 collection (6–8 inches)							
Total carbohydrates	26.29	26.57	21.78	21.84	....	.....	6.78	6.61
Reducing carbohydrates	2.24	2.19	10.25	10.15	....	.....	0.44	0.38
Non-reducing	9.30	9.43	0.53	0.57	....	.....	0.63	0.67
Polysaccharides	14.75	14.95	11.00	11.12	....	.....	5.71	5.56
	July 8 collection (20 inches)							
Total carbohydrates	15.32	15.21	18.69	18.63	15.18	15.07	10.51	10.41
Reducing carbohydrates	2.85	2.54	7.15	7.20	5.00	5.10	Trace	Trace
Non-reducing	3.82	3.71	1.67	1.50	1.18	1.02	1.96	2.01
Polysaccharides	8.92	8.96	9.87	9.93	9.00	8.95	8.55	8.41

The percentage of the different carbohydrates of various organs estimated at different times throughout the growth period is tabulated in table III. Curves showing the changes in different sugar content in these organs are given in figs. 6–8. These curves show that in the roots the reducing sugars remain constant, while the

non-reducing sugars fluctuate throughout the season. This is just the reverse of what is found in the stems. In the leaves the

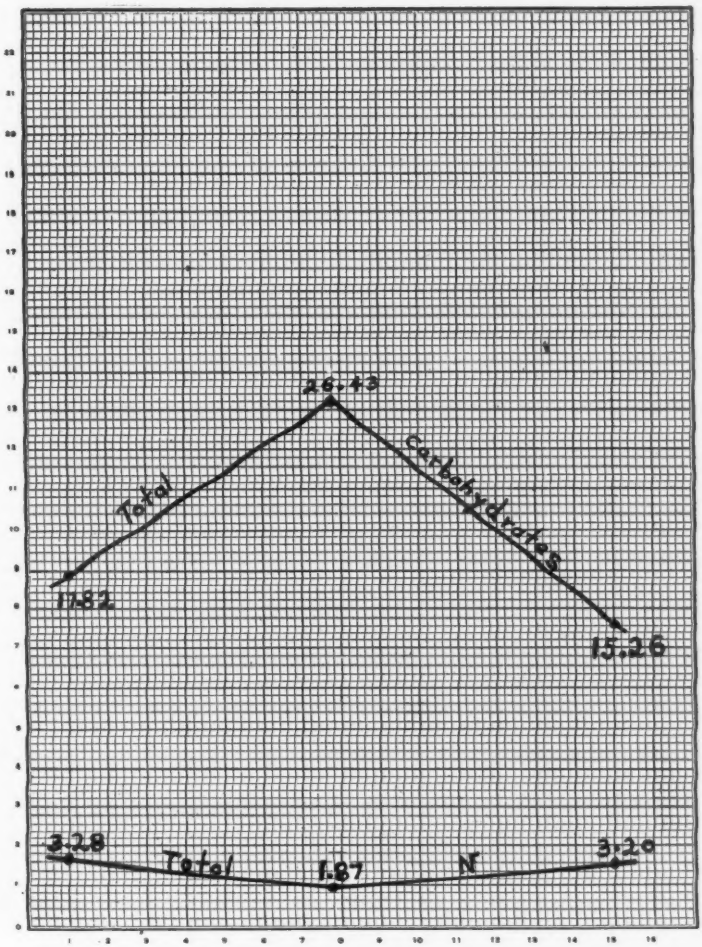

FIG. 9.—Reciprocal fluctuation of carbohydrate and nitrogen in roots (cf. figs. 3 and 6).

fluctuation of the reducing and non-reducing sugars is in the opposite direction; when the reducing sugars are high, the non-reducing

sugars are low, and the reducing type falls to zero at the time of seed formation.

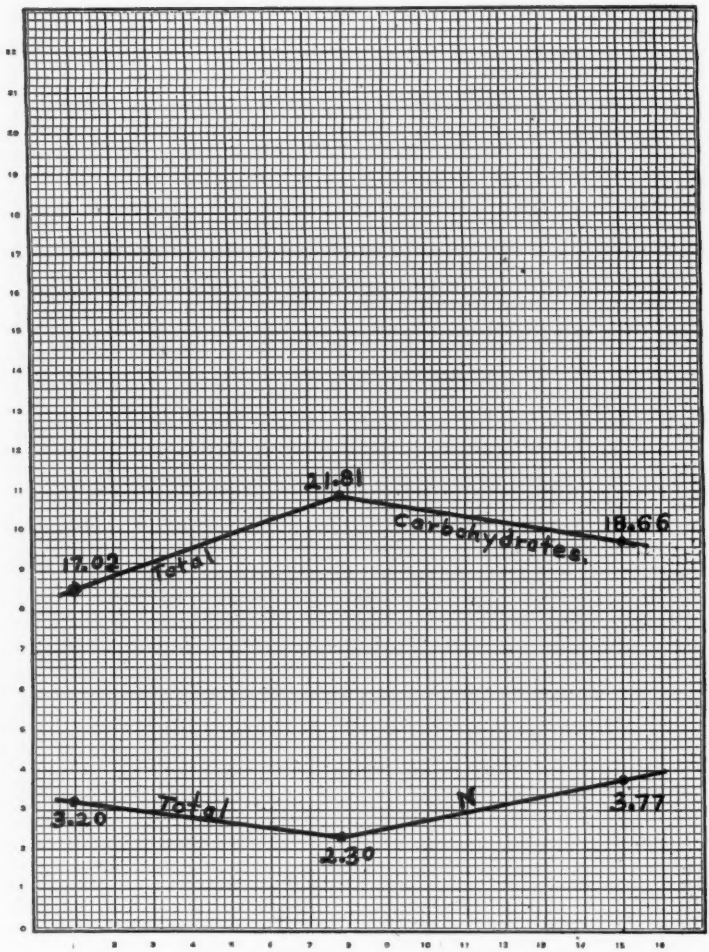

FIG. 10.—Reciprocal fluctuation of carbohydrate and nitrogen in stems (cf. figs. 4 and 7).

CARBOHYDRATE-NITROGEN RATIO.—According to the work of KRAUS and KRAYBILL on the tomato, high nitrogen in a plant is

accompanied by low carbohydrate. "Whatever the conditions under which a plant has been grown, considering the whole plant

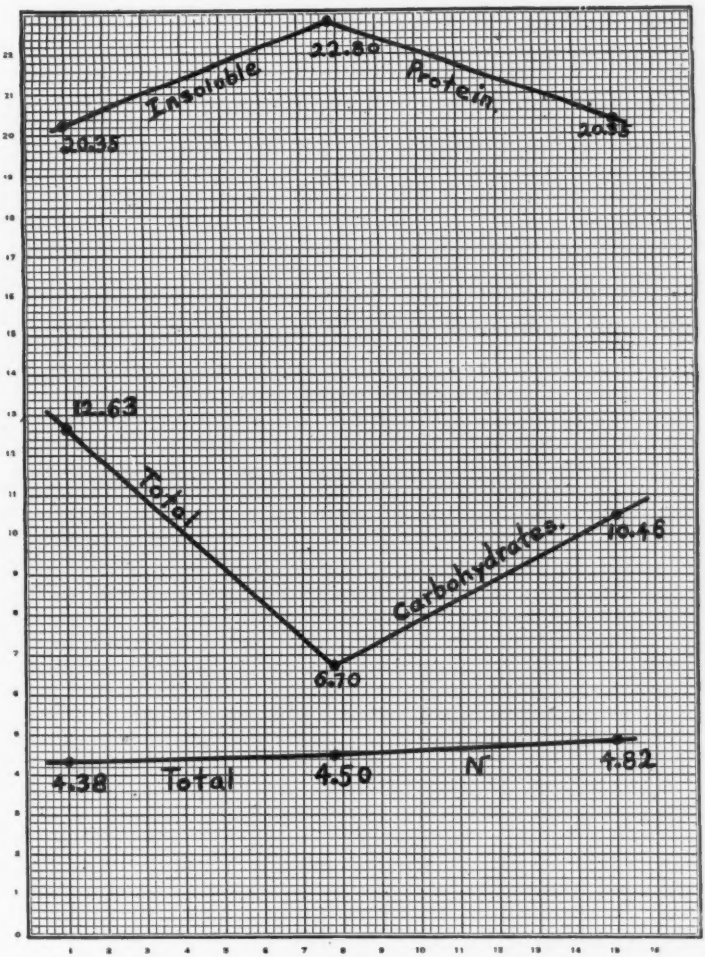

FIG. 11.—Reciprocal fluctuation of carbohydrate and nitrogen in leaves (cf. figs. 5 and 8); notice that carbohydrate reciprocates with insoluble protein and not with total nitrogen.

as a unit, increased total nitrogen and more particularly increased nitrate nitrogen are associated with increased moisture and

decreased free-reducing substances, sucrose, polysaccharides, and total dry matter."

In the work with *Amaranthus* plants I have found a similar situation so far as the relation between nitrogen and carbohydrate is concerned; that is, low nitrogen is accompanied by high carbohydrate and high nitrogen by low carbohydrate. Upon computing the reciprocal condition in the different fractions I find that the product of carbohydrate by nitrogen is not a mathematical constant, but that it varies considerably, sometimes decreasing as the development of the plant progresses. The product varies least in the stem and roots.

Let C_1, C_2, and C_3 be the carbohydrates and N_1, N_2, and N_3 denote the nitrogen, the sub-numbers representing the time of collection. If the carbohydrate and nitrogen hold a reciprocal relation, then $\frac{C_1}{C_2}=\frac{N_2}{N_1}$, $\frac{C_2}{C_3}=\frac{N}{C_2}$, and $\frac{C_3}{C_1}=\frac{N_1}{C_3}$; by clearing the fractions, $C_1 \times N_1 = C_2 \times N_2 = C_3 \times N_3$, etc., or carbohydrate × nitrogen = constant K. Applying this principle, the following constants are obtained.

Insoluble fraction (F_3)

	June 3	June 20	July 8	Av. K.
Roots	15.7	13.06	10.92	13.23
Stems	16.20	10.62	9.20	12.01
Leaves	28.00	20.4	27.6	25.30

Soluble fractions (F_1+F_2)

Roots	10.10	11.00	6.27	9.12
Stems	9.45	8.97	7.88	8.43
Leaves	2.39	2.97	3.15	2.84

These data show that the carbohydrate-nitrogen ratio is not a constant as we think of a constant in mathematics or physics. In plants where great fluctuation occurs in their substratum throughout different parts of the day and different times in the season, this disparity is no positive evidence that such a ratio does not exist. Secondly, regardless of the exactness of the ratio, this much is true, when the carbohydrates are high the nitrogen compounds are relatively low, and vice versa. Figs. 9–11 show this reciprocal

condition in the roots, stems, and leaves in the various stages of development.

SEEDS

A tissue analysis of the seeds was made in order to discover what important compounds were present and the distribution of these compounds in the various fractions. This gives one a more comprehensive knowledge of the chemical constitution of the plant.

PREPARATION.—The seeds were freed from chaff and cleaned in a breeze until all red seeds were removed and only plump black ones remained. These uniform seeds were then ground in a mortar with the pestle by taking a few at a time. A known quantity (25 gm.) was weighed out in triplicate for alcoholic digestion, and the tissues were fractionated and the carbohydrates, the nitrogen compounds, and the phosphorus determined.

TABLE IV

SEEDS; SUMMARY OF TOTAL CONSTITUENTS

Material	A. blitoides		A. retroflexus		
H_2O Av. 3	.....	9.45	.....	8.61	.....
Total N	2.55	2.42	2.54	2.47	2.37
Total P	4.01	3.93	4.63	4.60	4.65
Total carbohydrates	48.27	48.43	47.22	47.03	47.37
Lipins	4.58	4.44	7.67	7.86	7.78
Ash (ignition)	3.50	3.68	4.27	4.19	4.14

DIFFERENT FORMS OF PHOSPHORUS.—After separating the seeds into fractions F_1, F_2, and F_3, the different phosphorus compounds were estimated in the three fractions. In each case the phosphorus was determined by the Pemberton-Kilgore method (10). The analysis of the different forms of phosphorus is given in table V, and the percentage ratio of these different forms to the total phosphorus is given in table VI. The inorganic phosphorus was estimated by the CHAPIN-POWICK method (4). The amounts obtained from fractions two and three (F_2+F_3) were combined and calculated as total inorganic phosphorus. The soluble organic phosphorus was obtained by subtracting the inorganic phosphorus of fraction two only from total phosphorus in the same fraction (F_2). The

phosphoprotein phosphorus was split off from the insoluble fraction (F_3) by digesting a weighed amount (1–3 gm.) with 1 per cent NaOH solution at 37–40° C. for 48 hours in an incubator, after which the solution was neutralized with acetic acid and made up to volume. The insoluble material was separated from the filtrate by the use of the centrifuge. The filtrate obtained was tested for phosphorus with magnesia mixture. The magnesium ammonium phosphate precipitated out by standing overnight was dissolved, and the phosphorus was reprecipitated as ammonium phosphomolybdate and then titrated against a standard alkali (**8**). The nucleoprotein phosphorus was estimated by the difference between the total phosphorus minus the phosphoprotein and the inorganic phosphorus in the two fractions (F_2+F_3). The lipin phosphorus was determined by taking an aliquot of the ether soluble fraction (F_1). The ether was first driven off on a steam bath before acid digestion and the percentage of this phosphorus was estimated in the same way.

TABLE V

DIFFERENT FORMS OF PHOSPHORUS (PERCENTAGE P) IN SEEDS

Material	A. blitoides		A. retroflexus		
Inorganic P	0.133	0.137	0.131	0.123	0.132
Lipin P	0.014	0.013	0.019	0.020	0.017
Soluble organic P	0.011	0.012	0.023	0.033	0.034
Phosphoprotein P	1.240	1.340	1.840	1.950	1.790
Nucleoprotein P	2.610	2.430	2.620	2.470	2.670
Total P	4.008	3.932	4.633	4.596	4.649

TABLE VI

RATIO OF DIFFERENT P TO TOTAL P (PERCENTAGE P) IN SEEDS

Material	A. blitoides		A. retroflexus		
Inorganic P	3.32	3.49	2.83	2.65	2.84
Lipin P	0.35	0.33	0.41	0.44	0.37
Soluble organic P	0.28	0.31	0.49	0.72	0.73
Phosphoprotein P	31.00	34.10	39.74	42.40	38.50
Nucleoprotein P	65.30	61.80	56.60	53.70	57.50
Total P	100.25	100.00	100.07	99.91	99.94

Tables V and VI show the percentage of the different forms of phosphorus. The data in these tables show that about 96 per cent

of the total phosphorus is in the organic combination in the seed, existing (perhaps) as phosphoprotein and nucleoprotein phosphorus. Both of these forms are insoluble in water, alcohol, ether, and alcohol-water solvents. The inorganic phosphorus is relatively low, and the writer believes that the figures given for inorganic phosphorus in table V are even too high, because the greater part of the inorganic phosphorus was obtained from the insoluble fraction F_3 (4). Moreover, there is no proof that the reagents used did not break down some of the organic phosphorus. The lipin phosphorus is very low, varying from 0.014 per cent in *A. blitoides* to 0.019 in *A. retroflexus*, calculated on dry weight basis. It is interesting to know that in all cases the different forms of phosphorus are relatively higher in *A. retroflexus* than the corresponding forms in *A. blitoides*.

TABLE VII

DIFFERENT NITROGEN COMPOUNDS IN SEEDS (PERCENTAGE DRY WEIGHT)

Material	A. blitoides		A. retroflexus		
Total N	2.550	2.420	2.540	2.470	2.370
Nitrates NO_3	0.200	0.212	0.194	0.193	0.205
Amino N	0.096	0.095	0.089	0.090	0.090
Lipin N	0.027	0.027	0.031	0.032	0.033
Soluble proteins	2.260	2.270	2.660	2.790	2.890
Insoluble proteins	12.640	12.450	12.700	12.820	12.250
Total proteins	14.900	14.720	15.360	15.610	14.140

TABLE VIII

RATIO OF DIFFERENT N TO TOTAL N (PERCENTAGE DRY WEIGHT)

Material	A. blitoides		A. retroflexus		
Total insoluble N	83.20	82.00	80.40	79.00	77.00
Total soluble N	16.97	17.93	19.62	21.01	23.23
Total	100.17	99.93	100.02	100.07	100.23
Nitrate N	1.77	1.88	1.73	1.77	1.97
Lipin N	1.06	1.13	1.24	1.29	1.41
Amino N	4.68	4.65	3.50	3.66	3.84
Other soluble organic N	12.29	13.28	16.12	17.41	19.37

NITROGEN COMPOUNDS.—The distribution of nitrogen in the different fractions of the seeds is about the same as that of phosphorus. The insoluble nitrogen comprised 80–83 per cent of the total nitrogen (tables VII and VIII). The soluble fractions

contain only 17–20 per cent of the total, and most of it exists as organic nitrogen. The portion representing inorganic nitrogen is the nitrate nitrogen, which is relatively small. Calculated as nitrates (NO_3), the seeds contain 0.20 per cent, and this is equivalent to 1.80 per cent of the total nitrogen (tables VII and VIII). The lipin nitrogen is very small, only 0.027 per cent in *A. blitoides* and 0.032 per cent in *A. retroflexus*. These represent 1.10 and 1.31 per cent respectively of the total nitrogen content in these two seeds. In general a high percentage of insoluble phosphorus is accompanied by a high percentage of insoluble nitrogen, and a low percentage of soluble phosphorus by a low percentage of soluble nitrogen.

CARBOHYDRATES.—The polysaccharides are the predominating sugars in these seeds. *A. retroflexus* seeds contain 46 per cent and *A. blitoides* 47.75 per cent polysaccharides (on dry weight

TABLE IX

CARBOHYDRATES IN SEEDS (PERCENTAGE DRY WEIGHT)

Material	A. blitoides		A. retroflexus		
Lipin sugars	None	None	None	None	None
Reducing sugars	None	None	None	None	None
Non-reducing sugars	0.67	0.68	1.12	1.13	1.17
Polysaccharides	47.60	47.75	46.10	45.90	46.20
Total	48.27	48.43	47.22	47.03	47.37
	Ratio of different sugars to the total carbohydrates				
Non-reducing	1.38	1.40	2.37	2.40	2.47
Polysaccharides	98.65	98.60	97.60	97.60	97.60
Total	100.03	100.00	99.97	100.00	100.07

basis). If these sugars are calculated on the dry basis of the total sugars, the polysaccharides represent 97.60 and 98.60 per cent respectively in these two species. A striking contrast is seen on comparing the amount of polysaccharides in the green plant organs (figs. 6–8), which vary only slightly throughout the growing period, with that found in the seeds. In the growing period the highest percentage of polysaccharides was only 14.85, while that of the seeds was 47. In addition to this noticeable contrast, the soluble

sugars, both reducing and non-reducing, were comparatively high in the green plant organs (6–8 per cent), while those of the seeds are low (0.67–1.14 per cent).

LIPIN FRACTION.—The percentage of this fraction is 4.5 for *A. blitoides* and 7.78 for *A. retroflexus*. A closer examination of this fatlike substance shows that it contains phosphorus and nitrogen. The percentage of this nitrogen and phosphorus is very low (calculated on dry weight basis of whole sample). The presence of nitrogen and phosphorus indicates that the seeds contain phosphotides. The atomic ratio of nitrogen to phosphorus was determined by dividing the percentage by their respective atomic weights. The atomic ratio of N:P for *A. blitoides* is 1:2.3, and that for *A. retroflexus* is 1:2.6. This shows that other forms of phosphorus must be present than that existing in "ideal lecithin," which implies that the atomic ratio of N:P=1:1 (**5, 13**).

In addition to lecithin, a phytosterol was present in the seeds. This could not be quantitatively determined by using animal cholesterol as a standard, because animal cholesterol (**17**) has a different tint from that of the plant. BUCHARD'S color reaction gives a deep blue color for animal cholesterol, while for the seeds it gives a yellowish green. The amount of phytosterol in these two species of seeds was compared. Assuming that the phytosterol in *A. blitoides* is unity, that in *A. retroflexus* is 2.8.

TABLE X

CONSTITUENTS OF LIPIN FRACTION (F_1) OF SEEDS (PERCENTAGE DRY WEIGHT)

Material	A. blitoides			A. retroflexus		
Lipin, fats, etc. (wet basis)	4.15	4.07	4.23	7.02	7.18	7.09
Lipin (dry basis)	4.58	4.44	4.66	7.67	7.86	7.78
P in F_1	0.300	0.300	.....	0.240	0.260	0.220
P calculated in whole sample	0.014	0.014	.....	0.019	0.020	0.017
N in F_1	0.600	0.640	.....	0.402	0.400	0.420
N calculated in whole sample	0.027	0.027	.....	0.031	0.031	0.033

INORGANIC ELEMENTS.—The 1917 seeds of *Amaranthus retroflexus* were used for the estimation of the inorganic elements. The percentage of total ash given in table IV is 3.59 per cent for *A. blitoides* and 4.20 for *A. retroflexus*, but this is actually too low

for the inorganic elements present in the seeds, because the total phosphorus alone is 4.0 and 4.60 per cent respectively for the two species. This discrepancy is due perhaps to the loss of nitrates and part of the sodium, potassium, etc., in burning in the electric muffle. In the following analysis of the inorganic elements acid digestion (concentrated H_2SO_4+concentrated HNO_3) was used for the kations and alkaline fusion for the anions. The potassium was determined directly in the presence of all other elements except ammonia (NH_4−ion) and strong acid, as $K_2NaCO(NO_2)_6-\frac{1}{2}H_2O$ (11). Magnesium was estimated by the volumetric method as NH_4MgAsO_4 (7). The percentage of inorganic elements is as follows:

INORGANIC SALTS (*A retroflexus*); 1917 SEEDS

	Percentage	Percentage
Silica	0.42	0.40
$Al_2O_3+F_2O_3$	0.53	0.56
CaO	0.54	0.53
MgO	0.82	0.84
K_2O	0.38	0.35
Na_2O		no determination
Cl	Trace	Trace
SO_3	0.34	0.33
P_2O_5	8.90	8.85
N_2O_3 (nitrates)	0.12	0.123
Total (not including Na_2O)	12.05	11.98

Discussion

In spite of the inaccuracy of BOUTIN'S method for the determination of the nitrates, his results are probably not far from correct. He stated his results in percentage of KNO_3 as shown in the second column of table XI. I have calculated these as percentage of NO_3, as shown in the third column. His percentages of nitrates are not far from those found by me for the stems (8.57 per cent) and branches (12.50 per cent) of *A. retroflexus*, July 8 collection, as shown in table II.

	Percentage KNO	Percentage equivalent in NO
A. retroflexus	22.77	13.92
A. blitum	11.68	7.17
A. ruber	16.0	9.82

Table XI shows the results of the analyses of the seeds by various authors. In the main there is fairly close agreement, but in some cases there are considerable discrepancies. The discrepancies can probably be explained by the different chemical methods used by the various authors and by the lack of uniformity in the different crops analyzed.

TABLE XI

COMPARISON OF SOME OF THE ANALYSES ON *Amaranthus* SEEDS

MATERIAL	PAMMEL AND DOX		HARDING AND EGGE			WOO	
			A. retroflexus				
	A. blitoides	A. retroflexus	20 mesh	72 mesh	Oven dry	A. blitoides	A. retroflexus
Condition of seeds	.....	.....	Non-	uniform	.....	Matured uniform	Matured uniform
						Av. of 3	Av. of 3
H_2O	.....	.....	.....	11.28	8.60	9.45	8.61
Lipins (fats)	Little*	Little*	.....	7.92	8.46	4.56	7.77
Polysaccharides	Abundant	Abundant	39.77	40.98	44.83	47.68	47.21
Reducing sugars	.....	.....	Trace	Trace	Trace	None	None
Non-reducing sugars (sugar after inversion)	.....	.....	2.08	2.15	2.35	0.67	1.14
Nitrogen	1.88	2.49	.....	.....	.....	2.48	2.46
Protein	11.75	15.59	18.57	19.13	20.93	14.81	15.03
Ash	.....	.....	4.33	4.46	4.88	3.59	4.20

* Microchemical test.

From the results of this study it would seem that *Amaranthus retroflexus*, and probably other species of the same genus, can bear, as they ordinarily do bear, large amounts of free nitrates without being forced out of reproduction into extreme vegetation. This genus apparently is endowed with a very high capacity for nitrate absorption, as well as for maintaining its full seed production power in the face of a great excess of free nitrates. In this respect it seems to differ from the tomato studied by KRAUS and KRAYBILL, and probably from many other plants. Considering all angiosperms, it is likely that, due to hereditary characters, there is a great range of ease with which plants can be forced to excessive vegetation by extreme nitrate supply within the plant. It is well known that a given level of fertility that will throw small grains into extreme straw production with deficiency of grain will give excellent grain production in corn. This may be due to the lower nitrate absorbing power of the corn, to its greater

photosynthetic activity to balance the nitrates absorbed, or to the higher carbohydrate-nitrogen ratio accompanying best grain production. Which of these three possibilities really determines the situation can only be answered by such studies as those made or suggested on the tomato by KRAUS and KRAYBILL, or studies of the type made in this paper. It is evident, however, that there is need of numerous studies of the carbohydrate-nitrogen ratio in plants, both in regard to the factors affecting this ratio and the effect of the ratio on plant characters. As was suggested in the review of the literature at the beginning of this article, such studies are likely to throw much light on other physiological features than vegetation and reproduction.

Summary

1. There is a large amount of nitrate in the organs of *A. retroflexus*. The stem and branches are the primary nitrate storage organs. The rate of nitrate absorption increases with the aging of the plant, perhaps partly being due to the development of the root system with numerous branching rootlets, increasing the radius of the feeding area from a few inches to 2 ft. or more.

2. This high capacity for nitrate absorption and storage must be an important factor in making *Amaranthus* a very successful competitor against cultivated plants, so effectively withdrawing as it does the nutrient element most commonly limiting plant production. It would be interesting to know how generally and to what degree weeds possess this power.

3. The carbohydrates and nitrogen compounds fluctuate throughout the growing period. The fluctuation of the carbohydrates is in the reverse order of the nitrogen compounds. This inverse ratio is not a truly mathematical constant, but in general when the carbohydrates are high the nitrogen compounds are low, and vice versa. As the nitrate nitrogen composes more than 50 per cent in the stems and branches, there is a possibility that nitrates have some modifying effects on this reciprocal relationship. This inverse ratio is due partly to the synthesis of protein, chlorophyll, phospholipin, and other organic nitrogen compounds at the expense of the soluble carbohydrates.

4. Tissue analysis of the seeds shows the distribution of different forms of phosphorus in the various fractions. The organic phosphorus, which consists chiefly of phosphoprotein and nucleoprotein phosphorus, is high, and that of the inorganic form is low.

5. The distribution of nitrogen in seeds is in the same order as that of the phosphorus. The insoluble portion contains 80–83 per cent of the total. The soluble part varies from 17 to 20 per cent, most of which is in the organic form. The inorganic form is represented by the nitrate nitrogen.

6. The predominating sugars in the seeds are the polysaccharides. These compose nearly one-half of the total dry weight of the seeds. In both *A. retroflexus* and *A. blitoides* there is absence of lipin sugars in F_1 and reducing sugars in F_2. Only a small amount of non-reducing sugars was present in the two varieties.

7. The presence of nitrogen and phosphorus in the lipin fraction indicates that the seeds contain phosphatides. Phytosterol was also present. By comparison, *A. retroflexus* has 2.8 times as much as *A. blitoides*.

I am indebted to Drs. WM. CROCKER, S. H. ECKERSON, and F. C. KOCH for their kind advice and aid during the progress of the work.

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STAMINATE STROBILUS OF TAXUS CANADENSIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 255

A. W. DUPLER

(WITH PLATES XXIV–XXVI AND TWENTY-TWO FIGURES)

Introduction

In a previous paper (8) the writer described the gametophytes of *Taxus canadensis* Marsh., with the statement that other phases of the morphology would be treated in later papers. In this paper the staminate structures with respect to development and vascular anatomy are described. The lack of detailed information concerning these structures has seemed to the writer sufficient justification for the investigation here reported. In view of the generally recognized conservative character of the staminate structures in conifers, it seems that a more extended investigation of them, in the group as a whole, would be worth while. The description of the ovulate structures will be given in another paper.

The general statement in the previous paper as to material and methods will also apply here. The writer is under obligations to Professor W. L. EIKENBERRY, of the University of Kansas, for some material collected in northern Illinois a number of years ago. Acknowledgments are also due Professors JOHN M. COULTER and C. J. CHAMBERLAIN, under whose direction the study of *Taxus canadensis* was begun.

Historical

While the male gametophyte and its attendant features have received considerable attention, apart from the general more obvious features very little is found in the literature dealing with *Taxus* as to the morphology of the staminate strobilus itself. The earlier workers who studied the staminate structures of conifers were concerned largely in attempts to interpret them in terms of the angiosperm flower, naturally leading to confusion as to the true nature of the structures. These earlier views have been summarized by VON MOHL (26) in perhaps one of the most important

of the early papers dealing with the "male flowers" of conifers, and to which we are indebted for part of the following statement regarding this early interpretation of the staminate strobilus of *Taxus*.

LINNAEUS (16) regarded the entire strobilus as a single flower, with the stamens in a cylinder, the perianth lacking and replaced by bud scales. JUSSIEU (13) held that the strobilus was a monadelphous flower; while LINDLEY (15) considered the strobilus as a true cone with naked monadelphous flowers, each sporophyll representing a flower. RICHARD (19) went still farther, with the rather unique view that there were 5–8 flowers under each scale to which the stalk of the flower is attached on the underside. According to this view the pollen sac represented a "flower," and he had a similar interpretation for the sporophylls of *Thuja* and *Juniperus*. ZUCCARINI (28), regarding the reproductive structures as modified portions of the stem, comparable with the phylloclads of *Phyllocladus*, described the anther of *Taxus* as 7–8-lobed around the tip of a central column. He considered that *Taxus* has the most complete male flower in conifers, in other forms the anther folds growing on only one side of the central column, the other side growing out into a scale. VON MOHL opposes the idea of the stem character of the "flower" of conifers, and objects to the view that the "anthers" of other conifers have been derived from such a structure as that of *Taxus*, because "we have yet no certain data with which we can determine with certainty whether the anther of *Taxus* arises from one leaf or from a whorl of leaves."

As compared with our present ideas these early views are rather strange, having largely only a historical interest, with very little bearing on the real morphology of the structures concerned. Considerably later STRASBURGER (22) made some observations on *T. baccata*, describing the spiral arrangement of the scales of the strobilus and the grosser features of the development of the sporophylls. He held that the peltate stamen of *Taxus* represents the "extreme form of stamen," and found that it begins as a rounded knob about the first of August, becomes lobed by lateral swellings due to internal growth, and that pollen mother cells form in these lateral swellings and produce pollen by tetrad divisions. He also describes the pollen region as separated from the epidermis by two

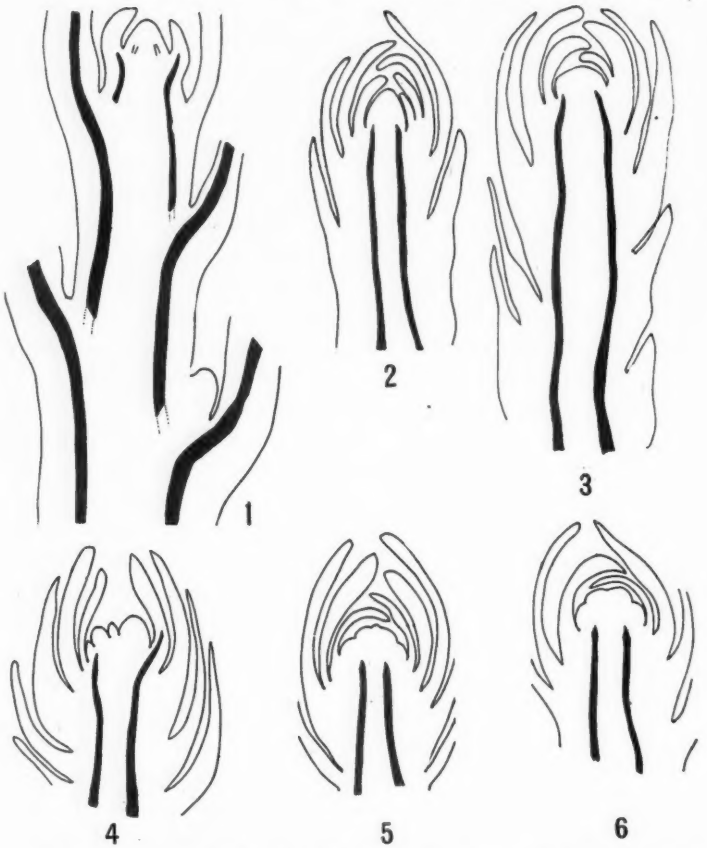
layers of irregular cells, and states that dehiscence is accomplished by the rupture of cells at the base and sides of the pollen sac. CHAMBERLAIN (3) describes the microsporangium of *T. canadensis* at the mother cell stage (October 1, 1897), at which time the nuclei are still rather small in comparison with the size of the cell, the tapetum being sharply differentiated, and its cells showing no tendency to plasmolyze like the cells of the sporangium wall. PILGER (18) describes the general external features, largely from the taxonomic viewpoint, speaking of the "flower" as consisting only of sporophylls surrounded at the base by a scale envelope which completely incloses the flower in the bud state. He regards the "leafy structure" of the anther, which is yet to be recognized in *Torreya* and *Cephalotaxus*, as being "entirely lost" in *Taxus*.

In the related forms the staminate structures of *Torreya* have been described in a general way by PILGER, based on *T. nucifera;* in more detail by Miss ROBERTSON (20) for *T. californica; and* by COULTER and LAND (5) for *T. taxifolia*. In *Cephalotaxus* some of the features of the spermatogenesis have been described by STRASBURGER (23), and by ARNOLDI (1). STRASBURGER (24) pointed out that the pollen grain divides in the sporangium before shedding; LAWSON (14) also confirms this in *C. drupacea;* and WORSDELL (27) gives a description of the general features of the "male flower," based on *C. Fortunei*, comparing it with those of other forms (*Phyllocladus* and *Ginkgo*), especially in the sporophyll features.

Strobili buds

In the axils of the leaves of the shoot of a given season 3 types of structures are produced: (1) the vegetative buds from which develop the lateral leafy shoots of the next season; (2) the young staminate structures, maturing the next season; and (3) the ovule-bearing structures, also maturing the next season. During the first season all of these structures are in bud form, the staminate buds during the latter part of the summer and winter being more globular than the other two kinds, which are so nearly alike in external appearance as to make their distinction uncertain except by very careful examination.

The rudiments of these structures begin to develop very soon after the beginning of growth of the terminal bud in the spring, the



FIGS. 1–6.—Longitudinal sections of young buds: fig. 1, young vegetative shoot with bud rudiment in axil of young leaf; fig. 2, vegetative bud with conical apex; fig. 3, staminate bud with broadened apex; fig. 4, ovulate bud, showing vegetative tip (to left) and rudiment of ovulate strobilus in axil of scale; fig. 5, young staminate strobilus, showing primordia of stamens and tip of axis; fig. 6, young staminate strobilus with primordia of sporophylls, axis apex not evident; ×36.

rudiments appearing as conical projections in the axils of young leaves (fig. 1). By the middle of June these axillary structures have

attained a length averaging about 1.5 mm., consisting of the main axis surrounded by compactly arranged scales. In this early stage one cannot distinguish these structures from one another, either by external appearance or in section. Early in July, however, one can recognize in median longitudinal sections the beginning of the differentiation which is now taking place, the apex of the vegetative bud remaining conical (fig. 2), as is characteristic of the vegetative stem tip (fig. 1), the apex of the staminate structure becoming broadened (fig. 3), while the ovule-bearing structure is recognizable by the rudiment of the ovulate strobilus appearing in the axil of one of the scales near the tip of the primary shoot (fig. 4). All 3 kinds of buds may occur on the same shoot; in fact, this is the usual occurrence, with the staminate buds generally the more numerous, the vegetative buds nearest the tip, and one to several ovulate buds a short distance below the vegetative ones, the staminate buds occupying the older portion of the shoot.

The buds arise only on the current season's growth, and in case of the staminate structures always mature the next season. No cases were observed in which staminate strobili were produced on older growth, nor were any cases found in which the buds remain dormant for a time and then mature. Miss ROBERTSON, in her study of *Torreya californica* from trees growing in England, found that while the staminate strobili are formed in the axils of the leaves of a current season, they may remain dormant for as long as 3 years. In *Taxus* buds may be found on older growth, but they are either dormant vegetative buds or persisting primary shoots of the ovuliferous structures of a former season, as will be described more fully in the paper dealing with these structures.

Sporophylls

PRIMORDIA

The broadened apex (fig. 3) is the first indication of the true nature of the staminate strobilus bud, and can be recognized first about July 1. STRASBURGER (**22**) was able to recognize the staminate structure of *T. baccata* about August 1, and in *Torreya taxifolia* COULTER and LAND (**5**) first observed the staminate buds in July,

but the primordia of the sporophylls did not begin to appear until August. The greater meristematic activity in some regions of this rounded apex than in others marks the position of the primordia of the sporophylls. These soon become rounded lobes above the general surface (figs. 5, 6). The nature of the growth of the primordium would indicate that it arises from a group of meristematic cells rather than from a definite initial; at least no defined sporophyll initial could be recognized.

The sporophylls are probably spirally arranged, although this is somewhat indefinite, and indications were found in a few cases that they may arise in acropetal succession (fig. 5); but if this is the case it is very soon obscured in the uniform development of the primordia as the sporophylls develop, no trace of the axis apex being recognizable after the very early beginnings of the sporophylls. The early development of the primordium is uniform in all directions from its central axis, at least until the differentiation of the archesporial initials takes place. The strobilus in this stage shows a series of rounded sporophyll primordia (figs. 5, 6, 23). The later development of the sporophyll is so intimately bound up with the development of the sporangia as to best be described in connection with them. In fact, the development of the sporangia determines the shape and character of the sporophyll, as aside from the sporangia the sporophyll consists of practically nothing excepting the short central axis and the epidermis.

MICROSPORANGIUM

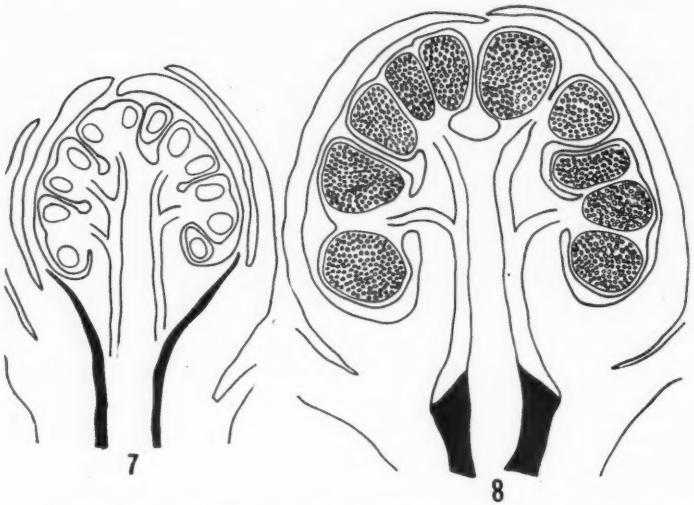
ARCHESPORIAL INITIALS.—HOFMEISTER (11) seems to have been the first to publish with reference to the microsporangium of conifers, reporting the spore mother cell stage as being reached in *Pinus maritima* in November. GOEBEL (9) traced the archesporium of *Pinus* to a single hypodermal cell, and claimed a similar origin for the archesporium of *Thuja*. His most important observation on this point was that the development of the microsporangium is like that of the eusporangiate ferns. COKER (4) in *Taxodium distichum*, and NICHOLS (17) in *Juniperus communis* var. *depressa*, also found a hypodermal origin of the archesporium, in the latter case consisting of "a plate of radially elongated cells, 4–6 in

number, when viewed in longitudinal section." COULTER and LAND, interpreting the structures in abortive sporangia, conclude that in *Torreya taxifolia* there is "a single hypodermal archesporial cell." Miss ROBERTSON did not get the origin of the sporogenous tissue in *T. californica*.

In *Taxus canadensis* the development follows the usual eusporangiate method, the 4–8 (usually 5–7) archesporial initials arising from the hypodermal layer of the sporophyll primordium while this structure is yet quite small (fig. 23), being uniformly distributed along its margin, and, dividing by periclinal walls, form the primary wall cell and the primary sporogenous cell (figs. 27–29), as in *Torreya taxifolia* (**5**) and most other forms. These initials are first to be recognized by the size of the cells and of their nuclei (figs. 23–26). One initial cell seems to be the rule, although cases were found in which the archesporium consists of 2 cells (fig. 26).

SPOROGENOUS TISSUE.—The primary sporogenous cell or cells soon divide periclinally (fig. 30) or anticlinally before or after the division of the primary wall cell, and by successive divisions the mass of the sporogenous cells is increased (figs. 31–35), the formation and growth of which result in the lobed peltate structure of the sporophyll, the sporangia being uniformly distributed around the central axis which continues the very short stalk of the sporophyll. As the tissue increases there is a corresponding growth of the epidermis and the sporangium wall (to be described later), the completion of which results in the separation of the sporogenous tissue from the other portion of the sporophyll (fig. 34). The tapetum is differentiated from the peripheral layer, and the remaining sporogenous mass increases in amount until the mother cell stage is reached early in October, as described by CHAMBERLAIN (**3**) and the writer (**8**). This has been given (**6**), and even quite recently (**7**), as the winter condition of the microsporangium, and has frequently been quoted by writers. As the author has already pointed out (**8**), microspore formation takes place during the early part of October, collections covering a number of years and from several localities in the northern United States bearing out the statement that the microspore is the winter condition of *Taxus canadensis*. STRASBURGER (**25**) found microspore formation in *T. baccata* taking place

in February in 1904, during unusually warm weather, indicating that in this form the sporogenous tissue remains in the mother cell stage until spring. It would be of interest to know the behavior in the extreme northern part of the range of *T. canadensis*, as it is possible that in regions farther to the north the microspore stage might not be reached before winter. The microspore mother cell stage is the winter condition of *Torreya californica* (20) in England



FIGS. 7, 8.—Median longitudinal sections of older strobili: fig. 7, at time of completion of sporangium wall, showing oval areas of young sporogenous tissue; vascular tissue of axis and upper scales, shown in black, embryonic vascular tissue of upper portion in outline; fig. 8, winter condition of strobilus, showing globular character of bud and microspores; vascular tissue as in preceding figure; ×36.

and of *T. taxifolia* (5) in Florida. During this development the strobilus has grown considerably in size (cf. figs. 7 and 8), becoming more pronouncedly globular, and it remains in this condition until the renewed growth of spring takes place.

No cases of abortive sporangia were found, and it seems a safe assumption that a sporangium develops from each initial or initial group. The adult sporangia show some variation in size, but not enough to indicate any tendency to abortion of any of them. This

is in marked contrast with the behavior in the related *Torreya*, in which a resin cavity results from the abortion of some of the potentially sporogenous tissue of the sporophyll, the abortion beginning at the primary sporogenous cell stage, as pointed out by COULTER and LAND for *T. taxifolia*. This results in the sporangia occurring on only one side of the otherwise peltate sporophyll. Miss ROBERTSON also finds that normally there are 4 sporangia on the side of the sporophyll of *T. californica* and a resin cavity on the other side, but that the strobilus axis sometimes terminates in a radially symmetrical sporophyll, like that of *Taxus*, with 6 or 7 mature sporangia. Whether a resin cavity is present in such a sporophyll is not stated, the inference being that most or all of the sporangium initials reached maturity. A similar abortion of sporangia, in the formation of mucilage cavities, is indicated by Miss STARR'S (21) work on *Ginkgo biloba*. COULTER and LAND find in *Pinus Laricio* resin cavities related to sporangia, exactly as are the lateral sporangia to the two middle ones in *Torreya*, and say "there is evident a tendency to reduce the number of sporangia by abortion, a reduction that has proceeded farther in *Pinus* than in *Torreya*, and in the latter farther than in *Taxus*." It seems that when resin or mucilage cavities are present in the sporophyll the sporangium initials are involved, and when absent these initials may all function normally, as in *Taxus*. Whether this can be made as a general statement for all forms with resin cavities in the sporophyll must wait for more extensive work on other forms. *Cephalotaxus* has a sporophyll similar in general appearance to that of *Torreya*, but it is not known whether any abortion takes place.

SPORANGIUM WALL.—The initial development of the wall is from the primary wall cell, which by a periclinal division forms a tier of 2 cells. As the sporogenous tissue develops, these wall cells divide anticlinally (fig. 31), increasing the extent of the wall layers. Only a portion of the wall is derived from the primary wall cell, however, as other cells abutting the young sporogenous tissue divide periclinally and add to the wall, first on the outer side (figs. 31–33) and then on the inner side as well (fig. 34), thus completely enveloping the young sporogenous tissue. The wall usually consists of 2 layers of cells, although 3 or even more layers may be present,

especially at the angles formed by the mutual pressure of the sporangial lobes.

As the sporogenous tissue increases in size there is pressure upon the wall cells, and they become flattened and extended, so that at the time of their maximum development they are broad thin plates. By the time of spore formation they are usually quite flattened (fig. 36), and during the further growth of the spores become more or less disorganized, so that by the time the spores have reached maturity, just before shedding, the wall has become a very thin layer abutting the epidermis, which has now become, in effect, the functional sporangium wall.

TAPETUM.—At the time when the sporangium wall is completed the sporogenous tissue inclosed within it is uniform in appearance (fig. 34). Soon, however, the peripheral layer of this tissue becomes differentiated as a tapetum (fig. 35), thus originating from the sporogenous tissue and not from the inner layer of the sporangium wall, as in some forms. The tapetum, however, has its chief significance from a physiological standpoint, being generally regarded as a nutritive layer, its origin seeming to be of little morphological significance. The tapetal cells are usually uninucleate, but not infrequently are binucleate (figs. 35–36). The tapetum is quite distinct during later phases of the development of the sporogenous tissue, is sharply differentiated at the spore mother cell stage, as pointed out by CHAMBERLAIN (3), and remains distinct during the early winter (fig. 36). With the growth of the microspores in the spring it becomes less and less prominent, until near pollination it consists of only a very thin layer of disorganized material surrounding the spore mass.

EPIDERMIS

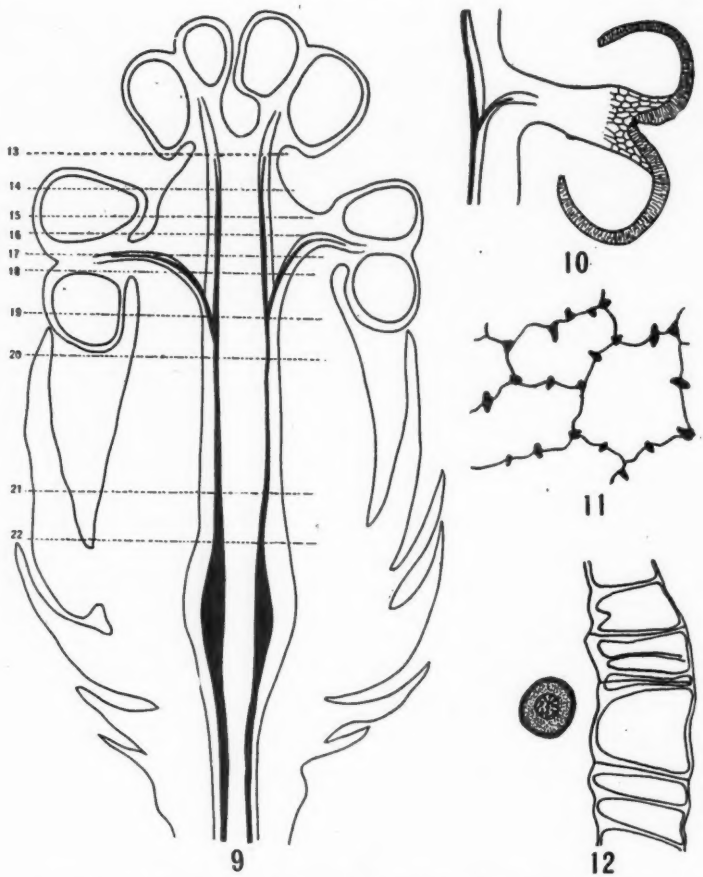
From the beginning of the primordium to the mature sporophyll the epidermis remains as a distinct layer, the sporangium developing from hypodermal tissues, as already stated. During the early growth of the sporophyll the epidermis is meristematic throughout, dividing anticlinally (fig. 24), its surface area thus keeping pace with the increase in the mass of the sporogenous tissue. An occasional periclinal division results in the epidermis becoming 2 cells thick at

some points. The meristematic ability, however, soon becomes limited to the base of the sporangium, the epidermal cells of the remainder of the sporophyll becoming larger and with less dense contents, the cells at the base remaining isodiametric and rich in cytoplasm (figs. 32–34). As the sporangium increases in size, causing a more pronounced lobing of the sporophyll, the necessary increase in epidermal surface is effected by the enlargement of the non-meristematic cells and the addition to them of cells from the basal meristematic region. The enlarged cells become filled with an amorphous substance and the walls become thicker.

By the time the sporangia are mature the epidermis has become the functional wall of the sporangium, owing to the practical disintegration of the true sporangium wall. At maturity the epidermal cells are devoid of contents and have the markings characteristic of the walls of many sporangia (figs. 11–12), these thickenings exercising a hygroscopic effect, useful in the liberation of the spores. JEFFREY (12) regards this thickening of the epidermal cells of the sporophyll, in a mechanical dehiscing device, as the result of the invasion of the epidermis by mechanical tissues of fibrovascular origin. There are no indications in *Taxus* of mechanical elements elsewhere in the sporophyll. COULTER and LAND found numerous stomata in the epidermis of *Torreya*. In *Taxus canadensis* there is a single stoma on a sporophyll, at the center of the peltate disk, occupying the bottom of the depression caused by the enlarged sporangia (fig. 10). GOEBEL (10) shows a similar situation in *T. baccata*.

Mature strobilus

The scales at the base of the strobilus are small and decussate, increasing in size and becoming spiral in arrangement above, the uppermost ones being considerably larger than the lower ones, and function as bud scales in the immature condition of the strobilus. The scales are brownish in color, with heavily cutinized outer epidermal walls, especially on the abaxial surface, the stomata occurring only on the inner surface (fig. 37), reversing the condition on the vegetative leaves of the plant, where the stomata occur only on the lower (abaxial) surface. The midrib is marked by the



FIGS. 9–12.—Fig. 9, median longitudinal section of mature strobilus just before pollen shedding, showing "elongating region" of axis and 4 sporophylls; xylem of bundle black; note that xylem becomes centrally placed in upper portions and does not extend as far into stalks as phloem; numbers at left (13–22) indicate approximately levels of cross-sections of strobilus shown in figs. 13–22; fig. 10, median section of open sporophyll, showing elongated stalk, open sporangia, and solitary stoma in center of depression of disk; fig. 11, tangential section of mature epidermis, showing mechanical thickenings on walls; fig. 12, cross-section of mature epidermis, with microspore, at time of shedding; figs. 9, 10, ×36; figs. 11, 12, ×475.

vascular bundle, when present, and occasionally by sclerenchyma-like cells along the outer margin of the midrib. In the young strobilus the mesophyll of the scale is compact, but as the strobilus matures large air spaces develop. In addition to the solitary stoma found on the sporophyll, stomata occur on the strobilus axis between the bases of the sporophylls with rather surprising frequency, being found only on this portion of the axis and not on the portion between the upper scales and the lower sporophylls. While the functional character of these stomata might be open to question, owing to their position rather than to their structure, their chief interest probably lies in their morphological significance as hereditary structures from a more highly vegetative ancestral strobilus.

During the autumn, winter, and early spring the strobilus has the appearance of a globular "bud," the stamens being surrounded by the uppermost scales (fig. 7). The axis between the upper scales and the bases of the lower sporophylls is very short and remains so until a few days before maturity, during the latter part of April in central Pennsylvania, at which time there is a rapid enlargement and elongation of this portion of the strobilus, the effect being to push the sporophyll-bearing portion beyond the scales (fig. 9). A similar elongation of this region is reported for *Torreya californica* (20). COULTER and LAND described an enlarged pith region in the axis of the strobilus in *T. taxifolia*, which the authors suggest may be "an important storage region for the strobilus." No such enlarged region was found in *T. canadensis*. In addition to the elongation of this portion of the strobilus axis there is also an elongation of the stalk of the sporophyll (cf. figs. 9 and 10), resulting in the separation of the sporophylls from one another.

The sporangia do not hang freely from the underside of the disk, but are fused with the stalk on the inner side (fig. 9), and laterally are separated from one another only by thin partitions, the external furrows between the sporangia not extending all the way to the center, the sporophyll and sporangia thus constituting a very compact structure. RICHARD (19), STRASBURGER (22), and GOEBEL (10) gave accounts of the dehiscence of the sporangium of *T. baccata*, in which they pointed out the rupture of the sporangia at the base and the umbrella-like movement of the epidermal wall.

The process is the same in *T. canadensis*, the breaking of the thin-walled epidermal cells at the base of the sporangium in a circle around the base of the stalk, the rupture of some of the cells at the side of the sporangium, and the hygroscopic rôle of the thickened epidermal cells resulting in the wall of the sporangia spreading out in umbrella form, the thin partitions between the several sporangia also being broken in the process.

When young the strobili rudiments are erect in the axils of the leaves, but as they develop they become oriented in such a way as to hang pendent on the lower side of the shoot, the fertile portion of the strobilus being directed downward. GOEBEL (10) regards the position and the method of dehiscence such as to secure the most advantageous distribution of the pollen.

Vascular features

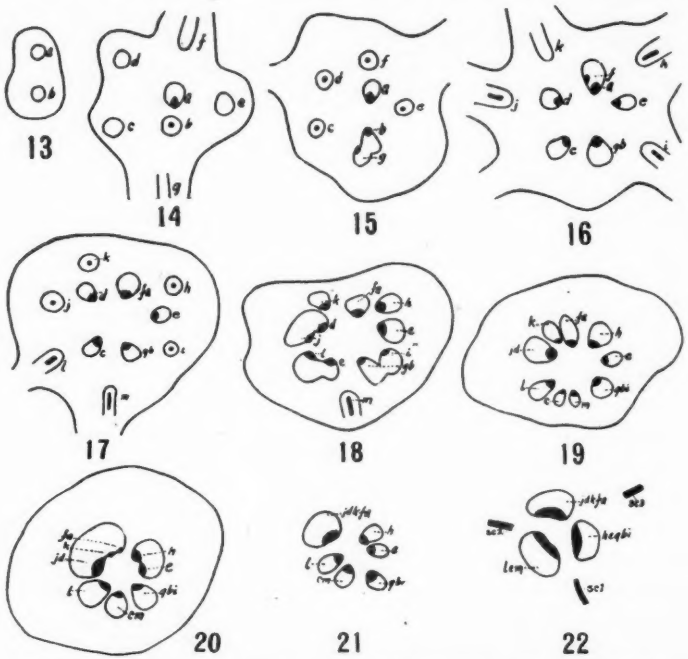
Since the reproductive organs, and especially the staminate structures, are regarded as among the most conservative of plant organs, a consideration of the vascular anatomy of the staminate strobilus is not without interest. While the ovulate strobili of conifers have been the subject of considerable investigation and discussion, in their vascular as well as in other features, the staminate strobili have not received much attention in their vascular anatomy, probably not as much as they deserve in view of the conservative nature generally assigned to them on other grounds. The only reference to this feature of *Taxus* is by STRASBURGER (22), who gave the arrangement of the scales of *T. baccata* and states that each stamen contains a bundle which passes into the stalk.

Like any other branch, the strobilus axis receives 2 bundles from the cylinder of the leafy shoot. These are semicircular in outline, and by meeting at their edges soon form a closed cylinder, broken here and there by the gaps formed by the weak bundle traces of the scales. In the lower portion of the strobilus, where the scales are small and decussate, the small traces often end in the cortex and do not reach the scale itself. The traces for the upper scales are better developed and extend for some distance into the midrib of the scale, especially in the 2 or 3 uppermost scales. Although the axis cylinder, as well as the cortical portion of the scale traces, are

collateral endarch, in their terminal portions they contain not only centripetal xylem, but are also accompanied by transfusion tissue which may be both dorsal and lateral to the xylem elements (fig. 38).

At the level of the uppermost scales the cylinder consists of 3 or 4 large bundles (figs. 22, 48) which extend into the fertile portion of the strobilus, where they branch, giving off finally a branch to each sporophyll, the bundle extending a little way into the base of the sporophyll stalk. In a young strobilus these bundles are represented only by elongated thin-walled elements, evidently procambium strands, which traverse the region between the base of the sporophylls and the level of the upper scales (figs. 7, 8). These strands remain in this embyronic condition until near maturity, when they elongate and take on their vascular features in connection with the growth of the "elongating region" of the strobilus axis. In this "elongating region" the pith becomes larger in diameter than in the lower portion of the strobilus, but shows no evidence of being in any way a storage region; in fact, there would be little use of a storage tissue at this stage in the development of the strobilus. The several large bundles of the strobilus axis extend for some distance into the "elongating region" and then give off branches to the various sporophylls, each of the large bundles supplying several sporophylls in this way (figs. 13–22). Some of the branches may unite and then separate (see the behavior of bundles *h* and *e*, and also of *l*, *c*, and *m*, in figs. 18–22), although usually the bundles pass rather directly to the base of the sporophyll (figs. 14–17, 44–47). Throughout the entire axis there is relatively a stronger development of the phloem than of the xylem, the latter forming a narrower zone than the former (fig. 48). Both xylem and phloem reach their greatest development near the level of the upper scales (figs. 9, 22), above this the xylem forming only a very narrow portion of the bundle. Throughout the strobilus the xylem consists of spirally thickened tracheids with bordered pits, the tracheids being rather short, however, although in the elongated region of the axis they are somewhat longer than at a lower level and the bordered pits are fewer in number. The phloem of the portion above the scales shows very little of the pitting present at a lower level, consisting

of elongated cells similar to those of the younger condition of the strobilus. Occasionally the xylem extends a short distance into the stalk of the sporophyll, the bundle here, however, usually consisting



FIGS. 13–22.—Cross-sections of mature strobilus at approximately levels indicated by numbers to left of strobilus shown in fig. 9; branches of bundles to various sporophylls indicated by *a*, *b*, *c*, etc., xylem indicated by black; bundles *a* and *b* supply terminal sporophylls; union of bundles indicated by combining letters, as *jd* in figs. 17–19; fig. 19 shows complete cylinder below lowermost sporophyll; in fig. 18 *l* and *c* united, in fig. 19 separated, in fig. 20 *c* and *m* united, and in fig. 22 *lcm* one of the 3 large strands from sterile portion of axis; fig. 22 also shows traces to 3 uppermost scales, *sc1*, *sc2*, and *sc3;* note concentric character of terminal portions, as in *c*, *d*, and *e*, in fig. 15; ×36.

only of the phloem portion (fig. 39), the xylem usually ending within the cortex of the axis.

The bundles of this region are collateral endarch in the lower portions. In the upper portions, however, the bundles frequently show centripetal xylem (figs. 40, 44), giving mesarch bundles, and

in some cases the smaller xylem elements are on the outside of the bundle, indicating a possibility of exarch structure (fig. 42). In addition, the xylem elements, in the terminal portions of the bundles, become more and more placed toward the center of the bundle, giving virtually a concentric bundle of a few xylem cells surrounded by the phloem portion of the bundle (fig. 41). No transfusion tissue was found elsewhere than in the scales.

Discussion

Perhaps the two most important features of the staminate strobilus of *Taxus* are the peltate sporophylls and the character of the vascular bundles of the scale and sporophylls. The peltate (epaulet) type of stamen occurred among the Paleozoic Cycadofilicales, in the *Crossotheca* forms, but the sporangia were bilocular and dehisced by a longitudinal slit along the adaxial face, the bilocular character being different from that of the modern gymnosperms. Peltate stamens are not known in Bennettitales, and none occur in the Cycadales. The peltate stamen has been carried forward to modern plants through the Cordaitalean line, in all probability, although so far as is known the stamens in the Cordaitales bore terminal erect sporangia. As COULTER and CHAMBERLAIN state, however, "it cannot be supposed that the stamens of so great a group were uniform in type," and it is very possible that peltate stamens occurred there also. The sporophyll of *Ginkgo* gives a suggestion of the peltate type of stamen, in occasionally having more than 2 sporangia, in the regular occurrence of more than 2 sporangia in fossil forms, and in the possibility, pointed out by Miss STARR, that the mucilage cavity replaces abortive sporangia. Among Coniferales there is a suggestion of the peltate stamen in the Araucarineae, and stamens of true peltate form occur in such forms as *Widdringtonia*, *Torreya*, and *Taxus*. In *Torreya* the true peltate character is generally obscured in the adult sporophyll owing to the development of the resin cavity from 3 of the 7 sporangium beginnings. Hence it is seen that peltate stamens, in one form or another, are scattered from Cycadofilicales to modern conifers, and there is no necessity of regarding such a sporophyll as that of *Taxus* as being of recent evolution. Assuming peltate

sporophylls in Cordaitales as probable, their continuation in Ginkgoales and Coniferales is quite possible, abortion of some of the sporangia in the formation of mucilage or resin cavities, in such forms as *Ginkgo* and *Torreya*, obscuring their true nature, but showing the true peltate character when all of the sporangia develop, as in *Taxus*. WORSDELL, following the view put forward by CELAKOVSKY (2), considers the peltate sporophyll of *Taxus* to have been derived from such a form as occurs in the Cordaitales, where the pollen sacs are "erect and terminal on the radial sporophyll," through such forms as found in *Cephalotaxus* and *Torreya*, where the pollen sacs are "sub-terminal and pendulous, owing to a slight prolongation of the axis of the sporophyll, between and beyond the sacs, in a small protuberance," this condition being intermediate between the Cordaitales situation and *Taxus*, "where the extended terminal portion has become enlarged and flattened out into a very distinct peltate structure." "*Taxus* thus represents an advance from the earlier types of *Cephalotaxus*, *Ginkgo*, etc., toward the subpeltate dorsiventral type of sporophyll of the true Coniferae." One must question the necessity of such an explanation for either the peltate sporophyll of the taxads or the dorsiventral one of most conifers, in view of the historical occurrence of both of these types in forms more primitive than even the Cordaitales.

The significant features of the vascular anatomy of the strobilus are the mesarch character of the terminal portion of the scale bundles, as well as the appearance of centripetal xylem in the terminal portion of the sporophyll bundle, where the bundle is not only mesarch at times, but may also be exarch and concentric. This indicates the very conservative nature of the staminate strobilus. These primitive features, however, occur only in the terminal portions of the strobilus, which may be regarded as an argument in favor of the "advanced" character of *Taxus*, compared with forms with more abundant centripetal xylem.

Summary

1. The staminate strobili occur in the axils of the leaves. The buds can first be distinguished from other types of buds by the broad apex.

2. The sporophyll primordia first appear as slightly rounded lobes above the general surface and may arise in acropetal succession.

3. The archesporial initials are hypodermal cells and develop according to the eusporangiate method. There are 4–8 of them, distributed around the margin of the primordium.

4. The sporogenous tissue reaches the mother cell stage about October 1, and forms microspores about 2 weeks later. There is no abortion of sporangia such as occurs in *Torreya*, the sporangia occurring in a circle around the stalk of the sporophyll.

5. The sporangium wall is usually 2-layered. The tapetum arises from the peripheral layer of the sporogenous tissue and persists until after megaspore formation.

6. The epidermis of the sporangium remains alive and thin-walled at the base, dehiscence being accomplished by the rupture of these cells at maturity, by the elongation of the stalk of the sporophyll. Owing to the disintegration of the sporangium wall, the epidermis is the functional wall in the later stages.

7. The strobilus matures the latter part of April. Just before maturity there is an enlargement and elongation of the axis, pushing the sporophylls beyond the scales.

8. The strobili of *Taxus canadensis* are somewhat smaller than those of *T. baccata*.

9. The strobilus bundles are collateral endarch, excepting in the terminal portions of the scale bundles and the sporophyll bundles, where they may be mesarch, and in the latter show indications of occasional exarch structure, the terminal portion of these bundles also being concentric.

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EXPLANATION OF PLATES XXIV-XXVI.

All drawings were made with a camera lucida; text figs. 1–10 and 13–22 are drawn to the same scale, with a magnification in reproduction of approximately 36; text figs. 11–12 and all plate figs. are drawn to the same scale, reduced one-half in reproduction, having a magnification of approximately 475.

PLATE XXIV

FIG. 23.—Young sporophyll primordium, showing 2 archesporial initials in hypodermal layer of rounded primordium.

FIG. 24.—Archesporial initial in hypoderm; division of epidermal cell.

FIG. 25.—Archesporial initial in tangential section.

FIG. 26.—Archesporium of 2 cells.

FIG. 27.—Metaphase in division of archesporial initial.

FIG. 28.—Late stage in division of archesporial initial.

FIG. 29.—Primary wall cell (outer cell) and primary sporogenous cell (inner cell), resulting from division of initial.

FIG. 30.—Primary wall cell and division of primary sporogenous cell.

FIG. 31.—Primary wall cell has formed 2 tiers of wall cells, in one of which division is taking place; primary sporogenous cell has divided anticlinally, forming 2 sporogenous cells.

FIG. 32.—Lobe of young sporophyll, showing small mass of sporogenous tissue, 2-layered sporangium wall formed on outer side, and meristematic basal portion of epidermis differentiated from remainder of epidermis.

FIG. 33.—Somewhat older stage than fig. 32.

FIG. 34.—Sporangium wall complete, entirely surrounding sporogenous mass; latter part of July.

FIG. 35.—Older sporangium, showing differentiation of tapetum from sporogenous tissue.

FIG. 36.—Portion of sporophyll, showing epidermis, 2-layered sporangium wall with narrow flat cells, tapetum (1 cell binucleate), and microspores; winter condition.

PLATE XXV

FIG. 37.—Transverse section of portion of lower scale, showing stoma on inner surface and heavily cutinized epidermal walls, especially on outer surface.

FIG. 38.—Transverse section of portion of upper scale, showing vascular bundle and inner epidermis of scale; in vascular bundle note centripetal xylem and 2 transfusion cells, 1 dorsal, 1 lateral to xylem.

FIG. 39.—Transverse section of bundle *a* of fig. 13, showing phloem character of bundle; no xylem present.

FIG. 40.—Bundle *a* at level of fig. 15, showing mesarch character.

FIG. 41.—Bundle *e* of fig. 15; single xylem cell surrounded by phloem.

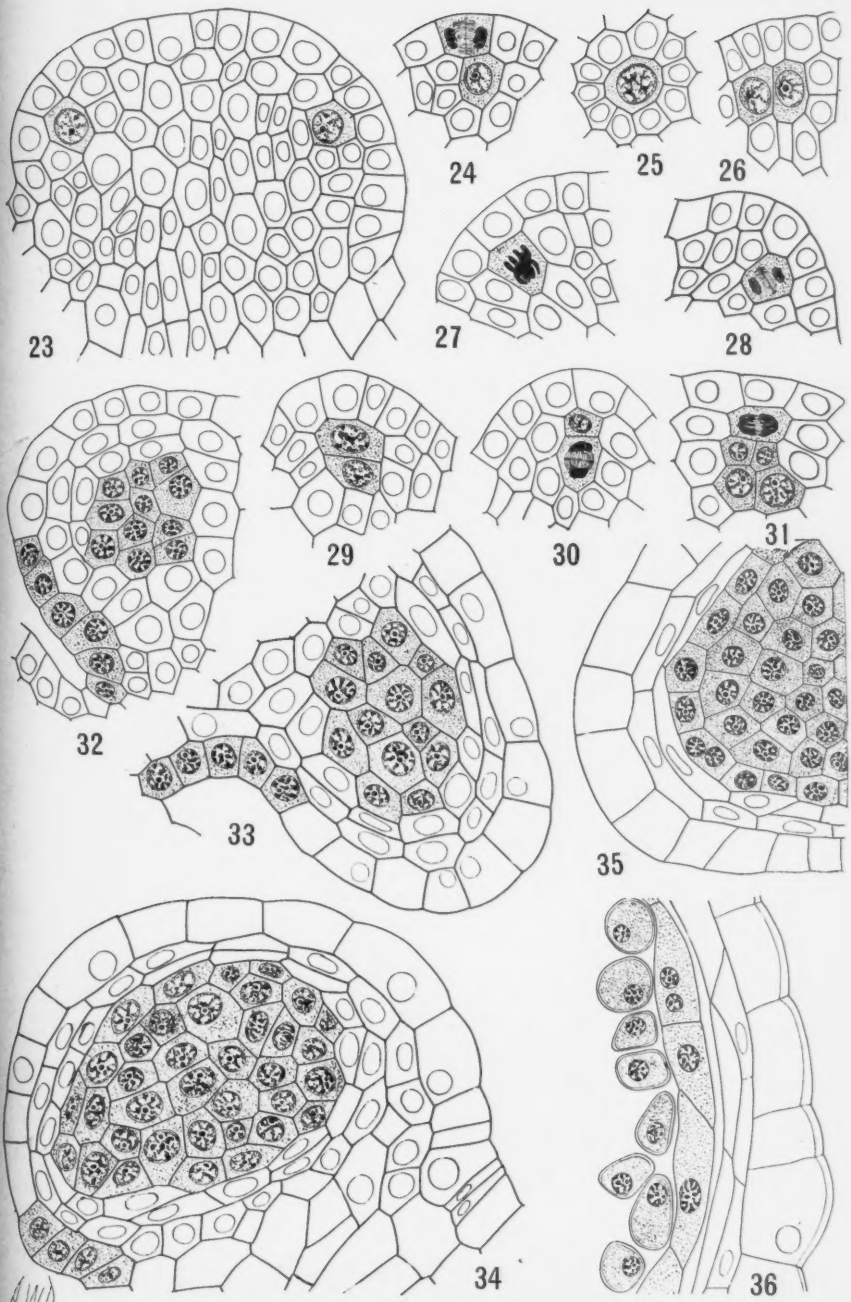
FIG. 42.—Bundle *e* at a lower level; large xylem cell centripetal to smaller ones, indicating possible exarch condition.

FIG. 43.—Bundle *gb* at level of fig. 17, showing collateral endarch character.

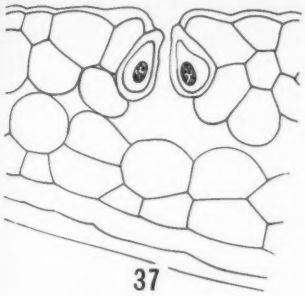
PLATE XXVI

FIGS. 44–47.—Fusion of bundles *f* and *a* (see figs. 15–17): in fig. 44 *f* is concentric, *a*, mesarch collateral; section 60 μ below level of fig. 15; fig. 45, enlarged view of bundle *fa* of fig. 16, 80 μ below fig. 44; fig. 46, 2 bundles near together 20 μ below fig. 45; fig. 47, fusion bundle *fa* 30 μ below fig. 46.

FIG. 48.—Transverse section of bundle *lcm* near level of fig. 22.



DUPLER on TAXUS



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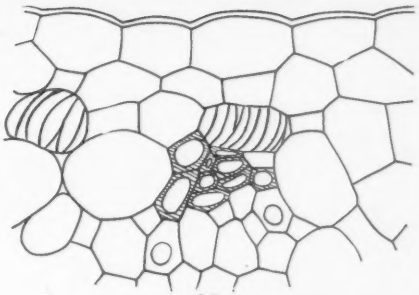

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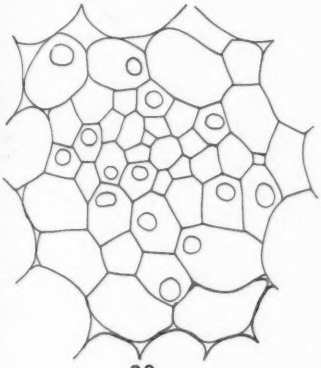

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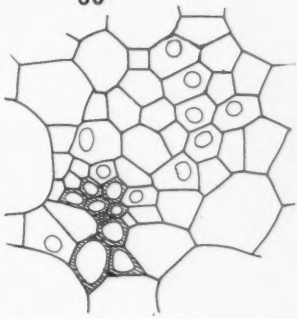

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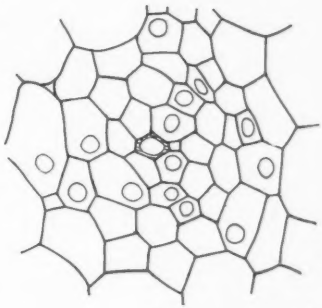

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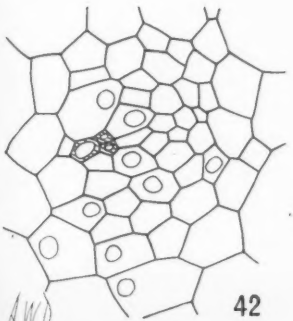

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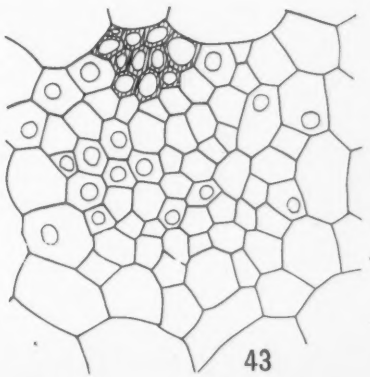

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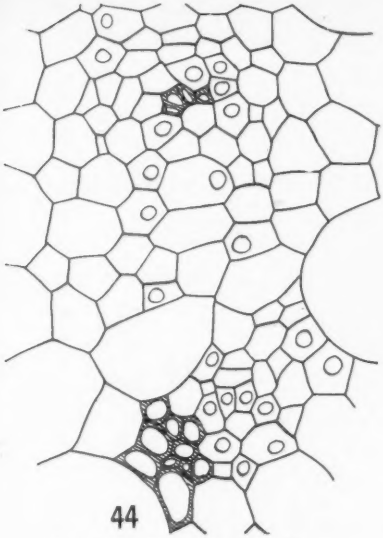
DUPLER on TAXUS



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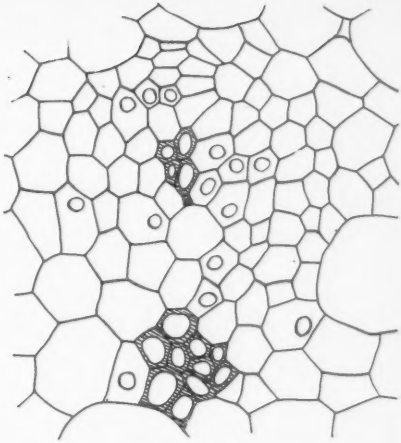

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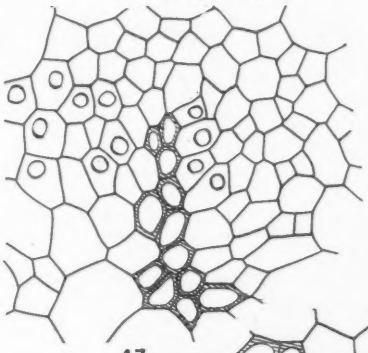

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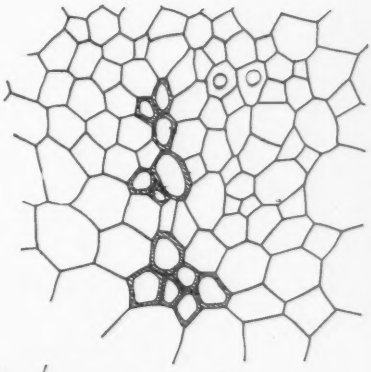

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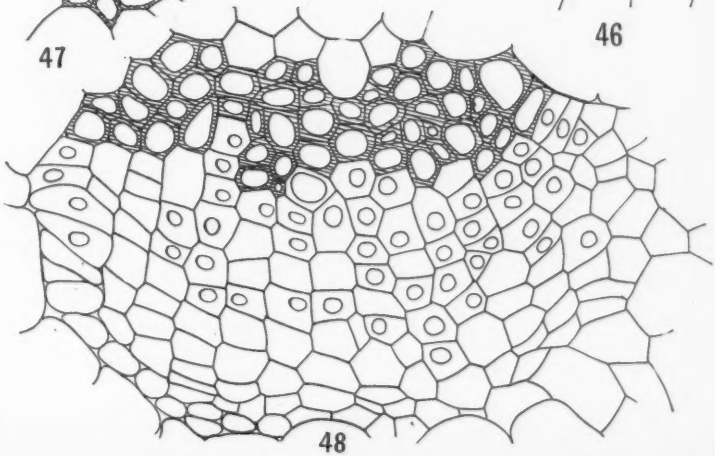

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A.W.D.

DUPLER on TAXUS

COLLOIDAL PROPERTIES OF BOG WATER

GEORGE B. RIGG AND T. G. THOMPSON

Introduction

This paper is a report of work on the chemical analysis of bog water, the colloidal state of the material in the water, and the effects of this material on the growth of plants. Much of the work is now reported for the first time, but a brief general statement of some of it has been made in a former paper (13). It is also shown how the data here given tend to explain current agricultural practice in bog utilization.

Sphagnum bogs are very numerous in the Puget Sound region and in Alaska. There is scarcely any portion of western Washington in which they are not found, and in some cases there are continuous areas of approximately 300 acres. The fact that these bogs act as a selective habitat and have a peculiar flora of their own, largely xerophytic, makes them objects of great botanical interest, while the fact that the substratum in them, often to a depth of 30 ft. or more, is composed almost entirely of organic matter, and that they have little or no forest covering, makes the utilization of these areas for agricultural purposes a matter of peculiar economic interest.

The acreage of these bogs now utilized for cranberry culture and for gardens, meadows, and pastures, although considerable, is still very small in comparison with their total area in the region. Every bog is a potential crop-producing area of considerable importance, and whatever we can learn with regard to the fundamental factors that govern the growth of plants in bogs in their natural state may function in their transformation into areas in which food production in this region can be increased.

In former papers the senior author has described the flora of some sphagnum bogs of the Puget Sound region (10) and Alaska (11), and has summarized and discussed the various theories (12) that have been suggested to account for the peculiar character of

the flora present in them and the almost complete inhibition from them of plants other than bog xerophytes. Among the various conditions which are factors in this, the toxic properties of the water have been shown to be very important. He has also (13) published evidence tending to show that the toxic properties of this water are due, at least in part, to the presence of matter in a colloidal state. The present paper gives fuller details of the experimental work pointing to this conclusion. It also reports further experimental evidence tending to support this conclusion and furnishes some evidence as to the colloidal state in which the toxic substances are present.

Experimental work

The samples of bog water used were collected in most cases by digging a hole, usually not more than 30 inches deep, in the substratum, and dipping from this the water that accumulated within a few minutes. When the bogs were too dry to admit of securing water in this way it was squeezed from handfuls of the decaying material. In all cases it was collected in glass containers and taken to the laboratory, where it was strained through washed cheesecloth and then filtered twice through filter paper.

Samples collected from several bogs and filtered in this way were treated with various electrolytes [NaCl, $MgSO_4$, $(NH_2)SO_4$, and Na_2HPO_4] to determine whether there was any material in the water that could be precipitated by this means.

To a 250 cc. sample from North Mud Lake bog a like volume of saturated solution of $(NH_2)SO_4$ was added. After shaking thoroughly this was allowed to stand. At the end of 2 days no precipitate had appeared, but at the end of 5 days a precipitate could readily be seen.

When 90 cc. of water from Fauntleroy bog was saturated with $(NH_4)_2SO_4$ by adding the salt gradually and shaking no precipitate appeared at once. When this had stood over night, however, there was a considerable quantity of precipitate, consisting of brown, somewhat flaky particles, some at the surface, some at the bottom, and some remaining suspended in the liquid. This precipitation by complete saturation with $(NH_4)_2SO_4$ was repeated

with two samples from the North Mud Lake bog and one sample from each of the following bogs: Maltby bog, Henry bog, and the bogs at Cordova, Alaska, and Sand Point, Alaska. The results in all cases were the same. The work was then repeated by saturating samples from these same bogs with NaCl, $MgSO_4$, and Na_2HPO_4. The results in all cases were the same qualitatively as when $(NH_4)_2SO_4$ was used, although the quantity of precipitate varied somewhat. Later precipitation of samples from these bogs and other bogs of the Puget Sound region was tried repeatedly, and in all cases the results were as described.

The precipitate from 7 samples treated with $(NH_4)_2SO_4$ was filtered off on filter paper. Each filtrate was then placed in a dialyzing tube of parchment paper and dialyzed in running water until the contents of the tubes showed no precipitate with barium chloride. The toxicity of these filtrates was then tested by growing cuttings of *Tradescantia* in them in the same way that the toxicity of bog water had been tested earlier (10). Controls of *Tradescantia* cuttings in untreated bog water and in Cedar River water were run. The root hairs developed well in the filtrate and in the Cedar River water, while their development was very poor in the bog water. No difference could be seen between those grown in the filtrate and those in the Cedar River water. Samples of bog water were also dialyzed and cuttings of *Tradescantia* were grown in them. They were still just as toxic as untreated samples.

On January 25, 1918, 6 samples of bog water that had been collected from Henry bog on November 5, 1916, and filtered through filter paper on November 7 and again on November 9, 1916, were all found to contain considerable quantities of a brown precipitate in irregular, somewhat flaky, masses. A sample collected from Maltby bog on November 30, 1915, and filtered December 4, 1915, showed the same results, as did also one collected at Sand Point, Alaska in July 1913, and filtered October 27, 1915. A gradual aggregation of the colloidal material occurred with the lapse of time in every case.

In order to test the volatility of the toxic substances, 500 cc. of the filtered bog water was distilled on a water bath until the residue was only 80 cc. The distillate was colorless, while the

concentrate was much darker than bog water. The material in the concentrate all remained in solution. No precipitate appeared and no incrustation was left in the beaker when the concentrate was poured out. The toxicity of the distillate and of the concentrate was tested by growing *Tradescantia* cuttings in samples of them. The concentrate proved to be more toxic than the untreated bog water, while root hairs developed as well in the distillate as they did in Cedar River water. Samples of the concentrate and of the distillate were saturated with solid $(NH_4)_2SO_4$ and allowed to stand overnight. The distillate gave no precipitate in any case, while the concentrate gave a much heavier precipitate than did the untreated bog water.

Since all samples of bog water tested had been found acid to litmus and to phenolphthalein, tests of both the concentrate and the distillate were made by titrating with N/20NaOH, using the same indicators. The acidity of the concentrate was in every case found to be greater than that of untreated bog water. The distillate was slightly acid, but much less so than the untreated water. The precaution of removing the CO_2 by boiling before titrating was taken in each case. Since both the concentrate and the untreated bog water are colored solutions, the use of indicators with them is unsatisfactory, and more exact means would have to be used in order to get quantitative data. Table I summarizes most of these results, together with some presented

TABLE I

Constituents	Precipitate with electr.	Toxicity	Acidity
Bog water	+	+	B
Distillate	−	−	C
Concentrate.....	+	+	A
Residue	+	+	B

later in the paper. The letters A, B, and C indicate relative acidity, A being greater than B, while C is much less than B.

Work was next undertaken to secure data on the chemical constitution of the materials in solution in the bog waters that had been experimented on and to get some indication as to what

the colloidal state of the toxic material is. Six samples of water were collected for analysis. The usual precautions recommended for the sampling of waters were rigidly followed. The localities were as follows: (1) the swamp adjoining Henry bog, (2) a point approximately in the center of Henry bog some 800 or 900 ft. from the edge of the swamp, (3) a point in West Mud Lake bog about 600 ft. from its edge, (4) another point in the same bog about 500 ft. from where sample 3 was obtained, (5) a very narrow strip of swamp land lying between the points where samples 3 and 4 were obtained, (6) a few inches under the surface of Lake Washington, a short distance from shore, where the water was about 30 ft. deep. These samples are referred to in table II by these numbers.

TABLE II

ANALYSIS OF WATERS FROM SWAMPS, BOGS, AND LAKES

Constituents	1	2	3	4	5	6
Total organic nitrogen	2.92	3.09	2.17	2.34	2.31	0.834
Nitrogen as albuminoid ammonia	0.855	0.850	1.20	0.526	0.500	0.20
Nitrogen as free ammonia	0.52	0.013	0.472	0.420	0.175	0.108
Nitrogen as nitrites	0.005	0.003	0.010	0.010	0.012	0.0002
Nitrogen as nitrates	0.05	0.05	0.040	0.040	0.030	0.06
Chlorine	0.96	1.44	0.708	0.743	0.702	2.70
Oxygen required	42.50	41.50	14.50	14.30	16.40	7.55
Total solids	109.00	101.20	92.00	93.00	107.00	65.00
Loss on ignition	83.50	85.70	62.00	65.00	64.00	20.00

Sample 1 possessed a strong earthy odor and a light straw color. It was collected so close to the edge of the bog as to be considerably influenced by the character of the water of the bog. Sample 2 had a very slight odor, and its color was much deeper than that of sample 1. Samples 3, 4, and 5 all possessed a slight earthy odor and had a light straw color. Sample 6 was perfectly clear and had no odor. Samples 1 and 2 were collected February 20, 1917; samples 3, 4, and 5 were collected March 13, 1917; sample 6 was taken during the spring of 1917. The various constituents of the waters examined were determined according to the methods given in "Standard methods for the examination of water and sewage," used by the American Health Association.

Determinations of the amount of solid matter and of the required oxygen on a few samples of water from other Puget Sound bogs gave results lying within the limits of the values reported for samples 3, 4, and 5. While only one analysis of Lake Washington water is here shown, the junior author has made frequent analyses of lake water in this region and has found them consistent with the analysis here given for Lake Washington water.

In table III the values in column 1 are an average of those in columns 2, 3, and 4 of table II. The values in column 2 are the same as those in column 6 of table II.

TABLE III

COMPARISON OF BOG WATER AND LAKE WATER

Constituents	1 (bog)	2 (lake)
Total organic nitrogen	2.53	0.0834
Nitrogen as albuminoid ammonia	0.855	0.200
Nitrogen as free ammonia	0.301	0.108
Nitrogen as nitrites	0.008	0.0002
Nitrogen as nitrates	0.043	0.06
Chlorine	0.963	2.70
Oxygen required	23.400	7.55
Total solids	95.400	65.00
Loss on ignition	70.900	20.00

FOULK (7) has made two analyses of Ohio bog water. A period of two years elapsed between the time of taking the two samples, which were from the same bog. The two analyses differ considerably, but both agree with the ones here reported in showing a large oxygen requirement and a large loss on ignition.

Several samples of Puget Sound bog water were evaporated to dryness in porcelain dishes on a water bath. A dark brown powdery residue was obtained. Other samples were evaporated to dryness in a porcelain dish heated over a Bunsen burner and protected only by a thin piece of asbestos and wire gauze. The residue so obtained was of the same appearance as that from the water bath evaporation. Some of the residue from the water bath evaporation was heated to redness in a porcelain dish. It gave no protein odor as it burned. The biuret test for protein

was applied to other portions of this residue. The results were negative in every case. The solubility of this residue was then tried. It dissolved readily in cold Cedar River water. Solution was complete in a volume of Cedar River water equal to the volume of bog water from which the solid matter was obtained. The toxicity of this solution of solids in Cedar River water was tested by growing cuttings of *Tradescantia* in it, with other cuttings in bog water and Cedar River water as controls. The bog water and the solution of bog solids greatly reduced root hair development on the cuttings, while root hairs developed abundantly on cuttings in Cedar River water. The water solution of this residue was found to reduce Fehling's solution slightly.

The brownish residue was found to be insoluble in alcohol and gasoline. In all cases but one it was also found entirely insoluble in ether. In this one case enough went into solution to impart a brownish color to the porcelain dish in which the ether was allowed to evaporate. When bog water was shaken with an equal volume of ether in a separatory funnel nothing was extracted from it.

An attempt was made to throw out the solid matter in bog water by centrifuging it. Samples were centrifuged for 20 minutes at 1800 revolutions, but no solid matter at all was thrown out.

Discussion

The experimental data furnish evidence as to the substances present in the water of sphagnum bogs, and also certain indications in regard to the colloidal state of the soil solution in them and the relation of this colloidal material to the toxicity of the water. They have also a considerable bearing on agricultural utilization of these areas.

The large amount of organic matter in bog water as compared with lake water is clearly shown by the large amount of solid matter and the loss sustained when this solid matter is heated to full redness. The solid matter in bog water is 146 per cent of that in lake water. The solid matter from bog water lost 74 per cent of its weight on ignition, while that from lake water lost only 30 per cent. That this organic matter is still in a very limited state of oxidation is indicated by the large amount of oxygen that

is required. More than three times as much oxygen was required by the bog water as by lake water.

The large amount of solid organic matter in bog water is evidently the result to a large extent of the breaking down of plant tissues in the absence of an adequate supply of oxygen. The interstices of the decaying mass of material are full of water at all times at a depth of 2 ft. or more, and even in the first 2 ft. except occasionally for a brief time in midsummer. Since organisms are abundant in bogs, it seems evident that the nature of the products must be conditioned by at least 3 factors: (1) the original composition of the decaying materials, (2) the organisms present, (3) the environmental conditions under which they act. DACHNOWSKI (**5, 6**) has emphasized the reducing power of bog soils, and has found that aeration lowers the toxicity of the water to agricultural plants. KLEIN (**9**) had made suggestions along this line as early as 1880.

A very large amount of the nitrogen present in bog water is in the organic form. The total organic nitrogen content of the bog water here reported is over three times as much as that of the lake water reported. In the bog water the total organic nitrogen is 50 times the combined nitrite and nitrate content, while in the lake water it is only 14 times.

The work of JODIDI (**8**) and of ROBINSON (**16**) show large amounts of nitrogenous matter in bog soils, and indicate that it is either already in the form of amino acids and acid amides, or is capable of being converted readily into these compounds.

The amount of nitrogen as nitrates is slightly larger in the lake water than in the bog water. Even if this difference were large it could not be accepted as necessarily indicating a difference between the nutritive value of the two waters for plants, since it is well known that some organic compounds are beneficial to plant growth (**2, 4, 17, 19**). A number of these beneficial organic compounds are nitrogenous. BOTTOMLEY'S (**2**) work indicates the abundance of such a compound in sphagnum peat that has been acted upon by aerobic soil organisms.

The amount of nitrogen as free and albuminoid ammonia varies considerably in the different analyses, and the existence of

nitrogen in these conditions is apparently influenced to a large extent by immediate local conditions.

KLEIN (9) was the first one to suggest that the toxicity of bog water is due to the presence of chemical combinations harmful to plant life. The water from a black bog overflowed into the meadows of a certain portion of East Prussia, causing considerable damage. He analyzed this water and found 312.8 parts per million of organic matter and 175.9 parts per million of inorganic matter. Among the reasons which he points out as to why this water is injurious to crop production these two are of special interest: (1) it acts as a reducing agent, (2) it produces chemical compounds that are harmful to plant life. The bog he studied differed from Puget Sound bogs in having considerable mineral content. Our bogs and his have in common the avidity of the organic matter for oxygen. His suggestion of toxicity is the earliest one seen by the authors. The review of his work says that when exposed to air the moist peat soon took up oxygen, with the result that there was formed on the surface of the soil a hard crust which was "impervious to the oxygen of the air, and the humus, withdrawing oxygen from the iron compounds, formed salts destructive to vegetable life." It does not seem positive from this wording whether the "salts destructive to vegetable life" came from the iron compounds or from the humus.

Previously published results (15) indicating that the osmotic pressure of bog water is very low had suggested that the material in solution in it is probably in a colloidal state. The data given seem to confirm this view, while the experiments with *Tradescantia* cuttings indicate that the matter in the colloidal state is a large factor in the toxicity of the water.

BAUMAN and GULLY (1) have shown that the acidity of bog water is due to the colloidal material of the cell walls of the hyaline cells of sphagnum. This would seem to suggest that both the acidity and the colloidal properties of bog water are due to the breaking down of sphagnum. The senior author has suggested in an earlier paper (10) that since the essential conditions for the formation of a bog are the continued growth of sphagnum and the

lack of drainage, the place to look for the origin of the toxicity is in the decay of sphagnum under anaerobic conditions. It seems probable (but is not proved) that the acidity, the colloidality, and toxicity all have their origin here.

It seems clear that the substances in bog water that are precipitated by electrolytes and on long standing without electrolytes, and will not dialyze through parchment paper, and, although present in considerable quantities, do not appreciably lower the freezing point of the water, are in a colloidal state. Since bog water and preparations from it (for example, the concentrate and the solution of the residue from evaporation) which contain these substances are toxic to *Tradescantia* cuttings, while preparations that do not contain them (the distillate) are not toxic, it appears that the toxicity is associated with the matter that is in a colloidal state. Concerning the nature of the colloid we have the following indications.

1. The colloid is thermo-stable. This is indicated by the fact that the distillate is non-toxic, while the concentrate is more toxic than untreated bog water.

2. The colloid is reversible, because when obtained as solid matter it is quickly redissolved on the addition of a volume of cold water equal to the volume of bog water from which the solid was originally obtained.

3. The solid material of bog water when obtained in the dry form is a granular powder. Since the distillate from which this was obtained as a residue is not colloidal, and since a solution having colloidal properties can be obtained by redissolving this solid matter, it seems that it is the material that is in the colloidal state.

4. Bog water has elsewhere (**15**) been shown to have a slightly lower surface tension than that of pure water. To what extent this may be due to the presence of the colloid has not been determined.

5. No means has been found of testing the swelling of this colloid, since it goes into solution so readily.

6. No evidence has been found that the colloids increase the viscosity of the water.

One means used in the Puget Sound region for bringing these bogs into cultivation is to drain them, scalp off and remove the living vegetation at the surface to a depth of 8 or 10 inches, and then stir up and aerate the partially decayed matter underneath. It seems probable that the success of this plan finds its explanation largely in the removal of much of the toxic material in draining off the water, and the oxidation of the remainder to non-toxic compounds when the soil is exposed to the air.

The work of BOTTOMLEY indicates that a substance or substances beneficial to the growth of higher plants and also increasing the rate of nitrogen fixation by soil organisms results from the action of aerobic soil organisms on sphagnum peat. To what extent the beneficial effects of the aeration of bog soils may be due to oxidation independent of organisms, and to what extent it may be due to the action of organisms under the changed conditions, have not been determined. It seems likely that crop plants in bogs that have been brought into cultivation are influenced beneficially by these compounds. To what extent this beneficial effect may result from actual use of these compounds as definite constituents of plant foods, or to what extent it may be due to their general catalytic effects or their part in certain definite types of metabolism, is not known. SCHROEDER (18) found that the direct application of peat alone to sandy soils gave increased yield of crops, although still better results were obtained when lime and stable drainings were used with the peat. Another practice for bringing these bogs into cultivation is that of destroying the surface vegetation by fire during the dry season. Usually the fire does not penetrate far into the sphagnum substratum because of the moisture beneath.

This practice results, of course, in the practically complete oxidation of the material in the surface layer of the bog. The preparation of the burned-over bog for the planting of crops and the subsequent cultivation of these crops secure aeration and consequent oxidation of the unburned material and also render the soil lighter by mixing the ashes with it. The acidity of the soil is neutralized to a considerable extent by the basic properties of the ash when it goes into solution.

In the main, however, the success that has been attained in the cultivation of bog lands in the Puget Sound region has been attained by the use of acid-tolerant crops (3) such as cranberries, strawberries, celery, onions, lettuce, cabbage, and carrots, rather than by correcting the acidity in order to grow crops that are not acid tolerant.

Summary

1. Bog water gives a precipitate on standing a few hours after saturation with electrolytes.

2. It also gives a precipitate on standing a year or more without electrolytes.

3. The filtrate from the precipitation with $(NH_4)_2SO_4$, when dialyzed until free from sulphates, is not toxic to the root hairs of *Tradescantia* cuttings.

4. Bog water, when dialyzed for the same length of time as this filtrate, is toxic to these root hairs.

5. The distillate from bog water gives no precipitate with electrolytes, is much less acid than bog water, and is not toxic to these root hairs.

6. The concentrate obtained when bog water is distilled to approximately one-sixth of its original volume gives a heavier precipitate with electrolytes than does bog water. It is also more acid and more toxic to these root hairs.

7. The residue from complete evaporation of bog water is a brownish powder which is soluble in cold water, insoluble in alcohol and gasoline, and practically insoluble in ether.

8. This water solution of the residue is toxic to the root hairs of *Tradescantia*.

9. No solid matter was thrown out of bog water by centrifuging.

10. Chemical analyses of Puget Sound bog waters give results similar to those reported for other American bog waters.

11. The toxicity of bog water to *Tradescantia* cuttings seems to be connected with the matter in it that is in a colloidal state.

12. The oxidation of this toxic matter to non-toxic matter seems to be a basis of agricultural practice in bringing bog lands into cultivation.

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VEGETATION OF UNDRAINED DEPRESSIONS ON THE SACRAMENTO PLAINS

FRANCIS RAMALEY

(WITH ONE FIGURE)

The observations here recorded were made in 1917 from March to May inclusive, chiefly in the neighborhood of Sacramento, California. Study, however, was extended in all directions for distances of 20 miles or more, and even to Chico, 90 miles north, and to Stockton, 40 miles south.

Most of the area is exceedingly flat and low. The city of Sacramento itself is at an altitude of 30 ft. above sea-level. Except to the east, in the Sierra foothills, there is scarcely a rise of 100 ft. within 25 miles. Soils are largely sand, sandy loam, sandy clay, and clay loam. Any fine grained soil which retains water is popularly known as "adobe," the term not being confined to clay.

The numerous shallow depressions[1] of the Sacramento plains[2] have arisen from the leaching out of limestone masses. They are of various extent, some only a few square meters in area and others 100 m. or more across, with a depth of a few centimeters or decimeters. The soil is very fine grained and holds water for an astonishingly long time after rains, often for one or two weeks. During the rainy period of winter these low places may be constantly full of water. Even with the lessened rainfall of early spring there is likely to be standing water during much of the month of March. Besides these vernal pools there are many shallow "draws," or ravines, having a vegetation cover which bears a resemblance to that of the undrained depressions. As would be expected, however, they have many more species of plants because

[1] These depressions could hardly escape the notice of botanists. Mention of their flora is made in the preface (p. 5) of JEPSON'S "Flora of Middle Western California," ed. 2. 1911.

[2] The term "Sacramento plains" is well established in botanical and popular writings, but these are not "plains" in the usual botanical sense. The vegetation is rather that of a vernal meadow or vernal prairie. During the growing period of March, April, and May the soil moisture is sufficient for the support of true mesophytic species; witness the abundance of European weeds.

of the more favorable edaphic conditions. The present paper will describe only the undrained depressions.

Early in March a spring outburst of flowering annuals occurs on the Sacramento plains. A conspicuous flora develops, which continues, with various changes, during two or three months. This vernal meadow-grassland shows great masses of color, due to the flowers of *Calandrinia*, *Eschscholtzia*, *Platystemon*, *Trifolium*, *Orthocarpus*, *Nemophila*, *Erodium*, *Lupinus*, *Gilia*, *Brodiaea*, *Layia*, *Lotus*, *Collinsia*, etc. Toward the latter part of May these annuals and the various grasses have ripened their seed, and the landscape has become brown.

The low places in the plains show no fresh vegetation for two or three weeks after the spring outburst takes place in the ordinary grassland, for the ground is still wet and cold. At 2 dm. the soil temperature is 12–15° C., instead of 18–21° as in the vernal meadow.

The earliest plant of the depressions is the "meadow-foam," *Floerkea Douglasii* (Limnanthaceae). This is a succulent herb about 1 dm. tall, with a profusion of white or roseate flowers. It shows first as a fringe at the margin of low areas, extending around on a contour line and resembling foam on a windy shore (fig. 1). Later, as the soil becomes drier and warmer, the meadow-foam works inward, meeting the vegetation that now appears in the central part of the depression. By May 1, at Sacramento, the meadow-foam has ceased blooming and has well developed fruits. Not all depressions have the fringe of meadow-foam here described, but very many do.

Two rather definite areas may be distinguished in the depressions. There is a central portion which remains wet for a longer period, and a marginal area around it which merges into the surrounding ordinary grassland. The central and marginal areas have their own characteristic plants. A condensed statement later in this article indicates seasonal differences in the appearance of the two areas.

Central area

The central area is characterized as a rule by a growth of *Allocarya californica*, a low, white-flowered borage, or sometimes by other species of the genus. Often the *Allocarya* forms a close

stand, with no other plants present.[3] The *Allocarya*, however, is sometimes scattered, and the chief mass of vegetation is "gold-fields" (*Baeria* or *Lasthenia*). Because of the almost constant occurrence of *Allocarya*, these plant communities might well be called "*Allocarya* depressions." The *Allocarya* continues in bloom until late in the growing season, even after the general spring aspect has passed. Many of the plants of this late period

FIG. 1.—A depression in early spring showing a wide circum-area of meadow-foam (*Floerkea Douglasii*); outskirts of Sacramento.

are markedly depauperate in vegetative parts, and also as to size of flowers; but even during the early part of the season depauperate individuals are scattered among the more robust plants.[4]

Gold-fields (*Baeria* and *Lasthenia*) sometimes replace almost completely the *Allocarya* of the central area. At a distance they

[3] The writer is familiar with such growths of *Allocarya* at Tolland, Colorado, in the Rocky Mountains along lake and stream margins in fine grained soil. The Colorado species in these places is *Allocarya scopulorum*.

[4] Many Californian species have depauperate or otherwise atypical individuals mixed with plants of typical form, or in some cases occurring in special habitats. It is often difficult to determine in a given case whether the differences are genetic or merely due to crowding or other environmental factors.

may be mistaken for some low-growing *Ranunculus*, because of the profusion of yellow flowers. These plants are found where there is some loose loamy soil above the close grained adobe, or where there is some drainage, as in a shallow draw. If the soil at the center of the depression is fine adobe, then the *Baeria*, if present, will form a ring outside the centrally placed *Allocarya*. Or, if water stands an unusually long time in the center, there may be a growth of *Damasonium californicum* (Alismaceae), bounded by a ring of *Allocarya*, and this in turn by a circum-area of *Baeria*.

Within the inner area of the depressions *Downingia*, a small Lobeliaceous plant, becomes conspicuous in late spring because of its great abundance. Even at a distance it may be recognized by its pale blue tint, for it sometimes fills nearly the entire central part after the flowers of *Allocarya* have disappeared. In some cases the *Downingia* develops as a wide circum-area, not quite reaching the center of the depression. Often associated with *Downingia*, and sometimes replacing it, is *Gilia leucocephala*, which when in large masses gives a whitish tinge to the vegetation complex.

"Coyote thistle" (*Eryngium Vaseyi*) is a prickly umbellifer with inconspicuous flowers which occurs in the central part of most of the depressions. Occasionally it forms a rather dense growth, but is more often rather loosely scattered. Hence, although it is the largest of the plants found at any time in the depressions, it has little influence upon the general appearance of the vegetation until late in the season, when it has grown tall and leafy, and when the showy annuals have completed their growth and dried up. The coyote thistle plants have a glaucous appearance that dominates the depressions, replacing the pale blue of *Downingia*, which followed the white of *Allocarya* or the yellow of gold-fields.

Among the less abundant plants of the inner area is the "freckled monkey" (*Mimulus angustatus* and *M. tricolor*). The plants are small, even minute, and top-heavy with large purple flowers spotted with yellow. When not prominent because of numbers the freckled monkey can nearly always be found rather late in April if search is made for it on the surface of the dried mud among the coyote thistles. Another small plant often abundant, and

practically always represented by at least a few specimens, is *Psilocarphus brevissimus*, a whitish-headed composite scarcely 1 cm. tall.

Marginal circum-area

The marginal zone is characterized in late spring by *Deschampsia danthonioides*. Sometimes this grass forms an almost pure stand after the disappearance of the meadow-foam, which, it will be remembered, marked the marginal zone in the first part of the season. As would be expected, the marginal zone is subject to invasion from within and from without, but it remains, as a rule, a very definite and distinct entity.

The inner boundary of the marginal circum-area is sometimes made by a thin line of *Achyrachaena mollis*, an inconspicuous composite that becomes noticeable in fruit by the spreading out of its silvery pappus. This same narrow line may be marked a little later in the season by a scattered ring of a kind of tarweed, *Hemizonia Fitchii*. Neither the *Achyrachaena* nor the tarweed, however, are essential elements in the circum-area; often they are entirely absent.

The ordinary drained areas of the Sacramento plains have a large proportion of introduced plants, especially grasses, bur clover, and species of *Erodium*. This is true of abandoned fields and of areas never under cultivation. The depressions, on the contrary, have an almost strictly native vegetation. If a patch of cultivated ground is left untouched, the depressions soon return to their original condition, unless the field has been thoroughly manured and cultivated. This recrudescence of the original vegetation, of course, is readily possible where most of the species are annuals. A definite case of return of native flora was noted in North Sacramento. An abandoned field had grown up to European grasses and weeds, but a low place in it, which plainly showed the marks of previous cultivation, was now like any of the primitive untouched adobe depressions. On May 15, 1917, the vegetation of the central area, about 10 m. across, was chiefly *Allocarya* and *Eryngium*, with a small amount of *Mimulus*, *Downingia*, *Gilia leucocephala*, and *Deschampsia danthonioides*. Outside this central part was a wide circum-area dominated at this time by *Deschampsia*,

with dried plants of meadow-foam, showing that the early spring aspect also had been of the usual type. Subordinate elements of the marginal ring were chiefly introduced weeds, *Silene, Lepidium, Hypochaeris, Rumex.*

The changing appearance of the depressions following the advance of the season may be shown best by a tabular statement.

PREVERNAL ASPECT

CENTRAL AREA.—Scattered plants of *Eryngium* left over from the previous season, but most of the ground bare.

MARGINAL ZONE.—*Floerkea* forming a more or less dense ring extending out to the ordinary grassland. The ring is often invaded toward the outside by species of the adjacent area, such as various native clovers, *Orthocarpus*, and European weeds.

MID-VERNAL ASPECT

CENTRAL AREA.—*Eryngium* actively growing but not yet of full size. *Allocarya* develops and blooms at the true beginning of spring; often forms a close community. *Baeria* and *Lasthenia*, if present at all, are likely to appear early. *Mimulus* sparingly present. Young plants of *Alopecurus* becoming abundant.

MARGINAL ZONE.—*Deschampsia* now showing definitely as a close growth of young plants. Native clovers in flower and fruit. Young plants of *Achyrachaena*. Introduced weeds.

LATE VERNAL ASPECT

CENTRAL AREA.—*Eryngium* now taller and more conspicuous than before. *Allocarya* continued from the previous aspect, but the plants now in bloom are depauperate. *Baeria* and *Lasthenia* in fruit or now drying up, with a few belated ones still blooming. *Alopecurus, Mimulus, Gilia leucocephala, Psilocarphus*, and *Downingia* in flower and fruit. In many places the last named very abundant and giving character to the entire depression.

MARGINAL ZONE.—*Deschampsia* in flower and fruit. *Downingia* scattered through the inner part of the marginal zone. Native clovers and *Orthocarpus* in fruit. *Achyrachaena mollis* in fruit.

At the outer boundary of the zone various introduced weeds now in flower and fruit.

Systematic list

The species of the depressions are recorded in the following list. Reference letters have the following significance: *C*, characteristic; *F*, frequent; *O*, occasional.[5]

Alismaceae

Damasonium californicum (F. and M.) Greene, *O*

Poaceae

Alopecurus californicus Vasey, *F*
Alopecurus geniculatus Linn., *F*
Deschampsia danthonioides Munro, *C*
Phalaris Lemmoni Vasey, *O*

Cyperaceae

Eleocharis acicularis R. Br., *O*

Juncaceae

Juncus tenuis Willd., *O*

Limnanthaceae

Floerkea Douglasii Baill., *C*

Lythraceae

Lythrum hyssopifolium Linn., *O*

Onagraceae

Boisduvallia stricta (Gray) Greene, *O*

Apiaceae

Eryngium Vaseyi C. and R., *C*

Polemoniaceae

Gilia intertexta Steud., *O*
Gilia leucocephala Benth., *C*

Boraginaceae

Allocarya californica (F. and M.) Greene, *C*
Allocarya stipata Greene, *O*
Allocarya trachycarpa (Gray) Greene, *F*

Lamiaceae

Pogogyne ziziphoroides Benth., *O*

Scrophulariaceae

Mimulus angustatus Gray, *F*
Mimulus tricolor Hartw., *C*

[5] The writer is under obligation to Professor HARVEY M. HALL for the privileges of the University of California Herbarium, and to both Professor HALL and Miss WALKER in naming for him a considerable collection of Californian plants.

Lobeliaceae

Downingia pulchella (Lindl.) Torr., *C*

Carduaceae

Achyrachaena mollis Shauer, *F*

Baeria chrysostoma gracilis (DC.) Hall, *C*

Baeria Fremontii Gray, *C*

Baeria platycarpha Gray, *F*

Blennosperma californicum (DC.) T. and G., *O*

Hemizonia Fitchii Gray, *O*

Lasthenia glabrata DC., *O*

Psilocarphus brevissimus Nutt., *C*

Cichoriaceae

Hypochaeris glabra Linn. (introduced), *F*

Summary

The numerous depressions of the Sacramento plains have a very fine grained soil, where water stands during the period of winter rain and even well into early spring. The vegetation is very different from that of the usual grassland of the region, being composed of very few species, with practially no introduced weeds. The depressions usually show a central area and a marginal zone, the former characterized by a dense growth of *Allocarya* or *Baeria*, and the latter by *Floerkea Douglasii* and *Deschampsia danthonioides*. Subordinate species of both areas are noted and the seasonal changes indicated. A systematic list is given of 29 species, 10 of which are marked as characteristic, 8 as frequent, and 11 as merely occasional.

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BRIEFER ARTICLES

AARON AARONSOHN

(WITH PORTRAIT)

The untimely death of AARON AARONSOHN in an aeroplane accident occurred May 15, 1919. He was on the postal plane from London, and the plane was wrecked in a heavy fog near Boulogne.

AARONSOHN is best known as the discoverer of wild wheat in Palestine, but his training and enthusiasm were expressing themselves in many ways, not only for the benefit of his own people, but also in helping to solve the general problem of food production. He was born in Roumania very shortly before his parents migrated to Palestine. He was educated in France, and then devoted himself to the agricultural problems and other interests of his home country. His discovery of wild wheat in 1906 was not an accident, but the result of a study of the problem, by which he became convinced that wild wheat would resemble cultivated wheat in appearance and size of grain. He found it growing in rock crevices, and later discovered that it is a relatively common grass throughout Palestine. A drought-resistant and disease-resistant race of wheat fired the imagination of AARONSOHN as to the possibilities of food production. His visits to the United States will always be remembered by those who met him personally or heard his addresses. He traveled throughout the country, investigating the agricultural

conditions, especially in the dry regions, and was convinced that the Palestine wheat would be of great service. Accordingly, in 1909, through the financial support of certain leading Jews in America, there was established in Palestine, at Haifa, the Jewish Agricultural Experiment Station; and in collaboration with the Department of Agriculture the breeding of suitable races of wheat was undertaken. The work of the Station dealt not only with cereals, but also with fruits, and was progressing with remarkable success when it was stopped by the war.

During the war AARONSOHN was absorbed by various activities, and he played an important part in taking General ALLANBY'S command across the desert into Palestine, and much of ALLANBY'S success was due to AARONSOHN'S advice and knowledge of the people and of the condition of the country.

In AARONSOHN'S death, at the age of 42, the science of plant-breeding, especially in its practical application in semiarid regions, has probably lost its most promising investigator.—J. M. C.

CURRENT LITERATURE

BOOK REVIEWS

Manual of American grape-growing

This book by HEDRICK[1] belongs to the Rural Manual Series edited by L. H. BAILEY, and, like the rest of the series, aims to cover the field outlined in an up-to-date way. The book is intended to furnish practical working directions to the grower of grapes, whether he uses the native varieties of the eastern regions or the *vinifera* types of the West. The economics of the crop, as well as considerations of soil requirement, vineyard management, and disease control, are presented with clearness, precision, and even with charm of style.

The chapters on breeding and domestication are given increased interest by a brief but comprehensive reference to the very unusual history of grapes and grape-growing in America. The general reader is likely to be surprised to learn how many of our standard varieties have been simply transplanted from the places where they were originally found as mutants or very favorable variations from type. The Concord, Catawba, Scuppernong, Herbemont, Isabella, and a host of other varieties are such products of unaided nature. The hard road traveled by those who in the early times strove to establish the European kinds in the East is lightly indicated. It would hardly be possible in a work of this character to deal more fully with the almost romantic, almost tragic, history of the early pioneers in grape culture. The several determined attempts to grow the European grape financed by various wine companies formed by public spirited men, by colonial governments, and by experienced refugees successful in the wine-growing regions of Europe were all doomed to lingering death, whether located in Pennsylvania, Virginia, or Kentucky. The full story of this phase of our grape history is still to be written.

The taxonomist may be disappointed to find the chapter on "Grape botany" limited to the 11 horticulturally significant species. The author, however, is probably right in tacitly considering the relations in the genus as a whole to be the property of the specialist in plant classification, although he complains of the hazy ideas of those who have thus far dealt with the group.

The book is up to the standard in its mechanical execution. It is abundantly supplied with fine half-tone plates of the most important varieties.

[1] HEDRICK, U. P., Manual of American grape-growing. New York: Macmillan Co. 1919. $2.50.

One may doubt, however, whether the cuts protraying the features of Prince, Rogers, and Munson flatter these leaders. As a whole, the book seems to do well what it is intended to do.—RODNEY H. TRUE.

NOTES FOR STUDENTS

Cactaceae.—It is probable that no monograph of a family is based upon more complete study than the monograph of Cactaceae by BRITTON and ROSE,[2] the first volume of which has just appeared. The illustrations are very numerous and admirable, and the colored plates are especially noteworthy. The authors began the study in 1904, and since that time the field work extended beyond North America, which was the original limit, so as to include the arid regions of South America as well. Those who are acquainted with the Cactaceae realize that not only are herbarium and field studies necessary, but also greenhouse studies, to discover the different phases that may appear during development.

The present volume includes the tribe Pereskieae, with its single genus *Pereskia*, represented by 19 species, 4 of which are new, and also the tribe Opuntieae, in which 7 genera are recognized, one of which is new (*Tacinga*). The large genus is *Opuntia*, with 264 species, grouped into 3 subgenera and 46 series, 32 of the species being new. New species are also described in *Pterocactus* (3) and *Nopalea* (2). All of the genera are illustrated, and of the 312 species 267 are represented by one or more illustrations. Of the 36 plates, 28 are in color.

The investigation has been financed chiefly by the Carnegie Institution of Washington, in cooperation with the New York Botanical Garden and the United States National Museum, while the United States Department of Agriculture has taken care of the living collections brought together in Washington. The completed monograph will consist of four volumes.—J. M. C.

Zinc and growth of Aspergillus niger.—STEINBERG[3] finds that he gets maximum growth in cultures of *Aspergillus niger* in flasks of Jena glass without additions of zinc sulphate, while less than half maximum growth is given in pyrex and Kavalier Bohemian glass flasks without zinc additions, and maximum growth with such additions (10 mg. Zn/L). STEINBERG thinks this is explained by the fact that Jena glass contains considerable zinc, while the other glasses do not. This accords with analysis of Jena and Kavalier Bohemian glass, and with the experiments of other workers on the relation of zinc to the development of this organism. He has also worked with 2 strains of *A. niger*, which he terms *W* and *Y*, and finds that the former demands a higher concentration

[2] BRITTON, N. L., and ROSE, J. N., The Cactaceae. Publ. Carnegie Inst. Washington 1: pp. 236. *pls. 36. figs. 302.* 1919.

[3] STEINBERG, R. A., A study of some factors influencing the stimulative action of zinc sulphate on the growth of *Aspergillus niger*. I. The effect of the presence of zinc in the cultural flasks. Mem. Torr. Bot. Club 17:287–293. 1918.

of zinc for its maximum growth than does the latter. He believes that the variation in zinc optimum found by different workers for this species can be explained in part by the difference in the strains, and in part by the difference in the composition of the cultural vessels used. He thinks that pyrex glass, if free from zinc, may bear other substances that stimulate slightly, and that gradual dissolution of these from the glass may account for the continual decrease in yield when cultures are repeated many times in the same flasks. He also admits that other unknown factors may account for this. He thinks it probable that this species has never been grown in total absence of zinc.—WM. CROCKER.

Multiple eggs in bryophytes.—FLORIN,[4] studying the archegonium of *Riccardia pinguis*, finds the axial row very variable. One archegonium contained an axial row of 4 cells, all of which had developed into eggs; another contained a single egg, 2 ventral canal cells, and 2 rows of neck canal cells; still another contained 4 eggs in the venter after the canal cells had completely disintegrated. Such so-called abnormalities are frequent in bryophytes, making it increasingly clear that both the antheridium and the archegonium are derived from a common gametangium, and that the archegonium occasionally reverts to that time when multiple eggs were the rule instead of the exception. Some mosses revert to a time still more distant, a time when both male and female gametes were present in the same gametangium, since we occasionally find both spermatogenous and oogenous cells in the same sex organ, which usually has the external form of an archegonium.—W. J. G. LAND.

Tyrosinase of fungi.—DODGE[5] has made a very careful chemical study of the action of tyrosinase on tyrosin. He obtained his enzyme from *Daedalis confragosa*, *Armillaria mellea*, and *Polyporus sulphureus*. He finds (1) that the tyrosin molecule is not deaminized, and (2) that in the formation of the colored compounds the tyrosin molecules are combined into larger molecules, accompanied by the masking of the carboxyl groups.—J. J. WILLAMAN.

Absorption of gold.—The ability of *Penicillium glaucum* and *Oidium lactis* to develop from conidia in colloidal gold solutions to which tannic acid or gum arabic has been added has been studied by Miss WILLIAMS.[6] The colloidal gold is slowly removed from solution during growth, removal being effected by the uncuticularized walls. The gold did not enter the protoplasm. No satisfactory explanation of the phenomena was found.—C. A. SHULL.

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[4] FLORIN, RUDOLF, Das Archegonium der *Riccardia pinguis* (L) B.Gr. Svensk. Bot. Tidskr. **12**:464–470. *figs. 4.* 1918.

[5] DODGE, C. W., Tyrosin in the fungi: chemistry and methods of studying the tyrosinase reaction. Ann. Mo. Bot. Gard. **6**:71–92. 1919.

[6] WILLIAMS, MAUD, Absorption of gold from colloidal solution by fungi. Ann. Botany **32**:531–534. 1918.